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FIFTH ANNUAL REPORT

of the work of the

CANCER LABORATORY

of the

York State Department of Health

Conducted at the

GRATWICK RESEARCH LABORATORY
UNIVERSITY OF BUFFALO

and

For the Year 1903-4

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TRANSMITTED TO THE LEGISLATURE APRIL 1, 1904

Albany
J. B. Lyon Company, Printers
1904

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REPORT OF THE DIRECTOR.

Buffalo, N. Y., *March* 17, 1904.

Dr. Daniel Lewis, *Commissioner of Health, Albany, N. Y.:*

Dear Sir.—I have the honor to transmit herewith the annual report of the Cancer Laboratory researches carried on under the auspices and by the aid of the Department of Health of the State of New York and in the Gratwick Laboratory of the University of Buffalo. The past year has not been characterized by any marked discoveries which can be safely announced to the world, but has been marked by steady, earnest and even laborious study along the lines of investigation, all of which are necessary and all of which are lending improvement in the study of cancer, either by chemical or biological means.

During the past year the staff of workers has included the following:

Dr. Harvey R. Gaylord	Pathologist.
Dr. H. G. Matzinger	Bacteriologist.
Dr. G. H. A. Clowes	Chemist.
Dr. Gary N. Calkins	Consulting Biologist.
F. S. Low	Assistant in Photo-chemistry.
W. S. Frisbie	Assistant Chemist.
Alice G. Owen	Assistant.
Clara A. Maclay	Secretary.
F. A. Payne	Janitor.
M. Weber	Laborer.
E. Kempkes	Laborer.

A portion of this time has been spent by Dr. Gaylord in Europe. Inasmuch as he was not working entirely in the interests of the Laboratory his salary was proportionately reduced. He has taken advantage of his presence in Europe to attend regularly the meetings of the Royal German Cancer Committee, of which, with myself, he is a member. He went to Copenhagen in the interests of the Laboratory to investigate the work of Jensen on the transplantation of carcinoma in mice. Professor Jensen was kind enough to give Dr. Gaylord some of his mice, which were brought to the Laboratory and used by Dr. Gaylord for the continuation and elaboration of the transplantation problem. A report from him is transmitted herewith. I also transmit herewith a paper by Prof. Gary Calkins, in which he has paved the way for still further announcements, of whose importance when made there can be no doubt.

One feature with which our study is more and more concerned is the variation of tumors of apparently the same general type, which are found to vary more and more, as do the personal features of different individuals of the same family. So marked is this variation that it becomes often confusing and makes it not only difficult to assign given specimens to their proper positions but even to indicate their relative degree of malignancy. Special attention has been given for the last few months to the study of tumors of the breast, in which Dr. Matzinger has been leader, and along the lines on which important theories have been advanced by Creighton in his remarkable new work entitled "Cancers and Other Tumors of the Breast."

The principal work of the chemical department in the course of the last year has been devoted primarily to the solution of the problem of obtaining immunity against cancer. Horses are being employed to prepare sera for use in incurable cases. Work of such a nature necessarily extends over long periods of time, and it will be impossible to give any final reports on this work in the immediate future. Experiments having a similar object in view are also in progress, making use of cancer mice kindly placed at the disposal of the Laboratory by Dr. Jensen of Copenhagen.

A great part of the time of the Laboratory staff has been devoted to work of a routine nature, several hundred analyses of stomach contents, urine, etc., having been carried out for the purposes of diagnosis. In several cases in which nutrition of patients has been seriously impaired systematic analyses of food and excreta have enabled us to materially improve their condition.

A part of the work undertaken by Dr. Clowes has been the chemical testing of the various staining fluids and materials used in order to better appreciate their reaction upon the cells constituting tumors, a subject on which there is much yet to be learned which will prove of value.

From the papers incorporated in this report and the abstracts of those published in the course of the last year in other journals, it will be seen that a considerable amount of work has been accomplished bearing on the physico-chemical problems of metabolism, the solution of certain micro-chemical problems and work of a serum-therapeutic nature.

The study of cancer everywhere provokes universal interest, evidences of which are seen in the increase of the activity of the medical journals devoted to this topic. A recent number of the British Medical Journal was almost exclusively devoted to the subject. There is now published in Germany, under the auspices of the German commission for the study of cancer, a *Zeitschrift für Krebsforschung* devoted exclusively to the subject, of which three numbers have already appeared. It proves to be a journal of great value. Such widespread activity is manifested in these various ways as to indicate the importance of this study to humanity.

In the last annual report I submitted a paper which had to do with applications, especially of the X-ray, in the treatment of cancer. In this paper I took the stand that it was too early to undertake to report results or do anything but gather material and watch. Now, a year later, I hardly feel justified in making any different statement, since while a large amount of material has been gathered and while men have watched eagerly, reports are so confusing and results so variant that it is still difficult to form a judicial estimate of their actual value. Recurrence has taken place in a few of the instances previously reported as cured and the whole question is still *sub judice.* I have had it in mind to summarize in a similar manner the subject of radiotherapy, but here the same confusion prevails. Opinions regarding this subject are discrepant. It is usually those who favor the use of the treatment, or those who have taken it up as a fad, who write the majority of the articles. Men who have been disappointed in its use or who have never become converted to it generally keep silent, and so their views are not heard.

Treatment of superficial growths by liquid air constitutes a method much more tangible, and, in some instances at least, promising. The conditions surrounding the method, however, make it impracticable.

Herewith is submitted a table, arranged by months, of the deaths occurring from cancer in the State of New York as taken from the Monthly Bulletin of the Department of Health. It shows an increase of about five hundred: that is to say, ten per cent, over the total number the previous year. How this figure can be explained on any basis other than actual increase I can not see. Certainly diagnostic methods have not so far improved within the year as to account for any such augmentation as this. It constitutes a rather startling corroboration of the estimate and even the prophecy contained in my first annual report of five years ago. I do not know what stronger argument could be advanced in favor of public assistance for the study of this disease than these figures furnished by disinterested observers.

TABLE I.

December, 1902	458
January, 1903	467
February	422
March	478
April	453
May	429
June	450
July	463
August	470
September	475
October	456
November	415
Total	5,436
For the year ending December 1, 1902	4,984
Increase	452

Below is submitted in Table II. a statement of the expenses of the Laboratory arranged as heretofore.

TABLE II.

Expenses of Conducting the Laboratory for the Period from October, 1902, to October, 1903.

1902.	Equipment.	Stock and material.	Sundry expense.	Salaries.	Total.
October	$100 81	$58 76	$90 15	$728 33	$978 05
November	97 48	195 95	105 54	762 33	1,161 30
December	40 10	95 91	224 48	809 83	1,170 32
1903.					
January	102 51	102 92	119 31	790 33	1,115 07
February	42 63	193 19	396 07	1,040 32	1,672 21
March	208 79	158 41	180 40	873 66	1,421 26
April	36 38	56 82	145 17	873 66	1,112 03
May	9 12	58 93	155 68	873 66	1,097 39
June	49 78	39 51	399 08	873 66	1,362 03
July	179 66	37 14	326 49	706 99	1,250 28
August	2 50	7 04	123 37	694 99	827 90
September	384 02	23 60	27 44	700 04	1,135 10
	$1,253 78	$1,028 18	$2,293 18	$9,727 80	$14,302 94

Of our appropriation of $15,000 for the year 1902-3, $651.32 was used before October 1, 1902, on account of the change in the fiscal year which made it necessary for the previous appropriation to stretch over twenty months; and we had on hand October 1, 1903, $45.74, which accounts for the full $15,000.

With regard to the needs of the Laboratory for the ensuing year, I can only say that it is contemplated to carry on the work about as previously. The appropriation heretofore, i. e., $15,000 per annum, just enables us to do this work, and I would respectfully urge a continuation of the appropriation of the same amount for the continuation of the work during 1904-5. I have observed no falling off in zeal and interest among those engaged in the work; on the contrary, each one connected with the institution has shown such a degree of interest and faithfulness as to be entitled to much public commendation. As before, I have to thank you again for the unfailing assistance which has been constantly rendered from your department and for the numerous evidences of your own personal interest and appreciation of the work done in Buffalo.

ROSWELL PARK.

THE CELL-INCLUSIONS IN CARCINOMA.

By Gary N. Calkins, Ph. D., Professor of Invertebrate Zoology, Columbia University, N. Y.; Consulting Biologist State Cancer Laboratory.

It is not my purpose in this report to drag in again the arguments for and against the parasite theory of cancer, nor to dwell upon the lines which such arguments have taken in the past fifteen years. My subject deals rather with the miscellaneous inclusions found in cancer cells and leads me to offer still another contribution to the already crowded literature upon the subject of these much contested structures. I do so not with the hope of saying a conclusive word upon this difficult topic nor of formulating a theory as to the aetiology of cancer, much less of announcing a "new cancer organism," but solely for the purpose of showing how these inclusions may be regarded by a student of cellular biology who has given particular attention to the structures of the cell as they appear in normal tissues and in the lowest animal forms.

It may be pointed out that biologists as a rule have given but scant attention to the cancer inclusions, the majority of them passing over the subject with little thought, or with some evidence of arrogance dismissing without study the characteristic structures as "more like technical artefacts than organisms." There has grown up, however, a great literature on the subject of organisms, especially protozoa, in cancer, most of which, to be candid, is unquestionably based upon a meagre knowledge of these lower forms of life. Assertions and generalizations have been made which not only betoken ignorance of protozoön morphology, but owing to their indefinite nature invite criticism from the biological side on the one hand and scepticism from the pathological side on the other.

The main contention which some writers have held that the inclusions are protozoa has been weakened by false analogies and comparisons and the facility with which such comparisons have been overthrown has discredited the majority of such attempts. On the other hand, a biologist attempting to interpret the varied structures in cancer cells invites the same form of criticism from the pathological side, and basing his conclusions on morphology alone, runs the risk of making equally false and unjustifiable assertions and generalizations. It is with the full conviction of this possibility of error that I offer in the following pages a critical review of the structures which I have independently observed during the time that I have been connected with this Laboratory.

Contrary to what appears to be the usual custom in studying cancer-cell inclusions, I have not examined a large number of tumors in support of any particular hypothesis. On the whole it seemed to me more profitable to study in detail a single case and to be familiar with all of the types of inclusion which that case might present. The advantages of this procedure are: (1) It makes possible the attempt to work out the relations of the different kinds of inclusions, to grade together those which are undoubted degenerations or secretions, and those which may be otherwise interpreted. (2) It presents all of the inclusions under the same conditions of the disease. (3) It permits a deeper analysis in a given time into the structural modifications of the particular type of cancer cells under observation. (4) It gives by reason of this more detailed study a better basis for comparisons with the inclusions in other types of tumor.

Although I have examined many different types of tumor more or less carefully, the one which I have studied in detail is designated as case 300D in the Laboratory records. It was a rapidly growing carcinoma of the breast, operated by Dr. Roswell Park in June, 1903. The report of the pathologist upon it is as follows:

On examination the tumor proves to be a soft solid carcinoma of the breast, in many places showing a distinct cystic character. The larger cystic cavities are lined with multiple layers of large epithelial cells, the nuclei and protoplasm of which stain deeply with haematoxylin-eosin. Karyokinetic figures are fairly frequent. In the smaller nests the membrana propria of the ducts is broken through and nests of epithelial cells can be found in the connective tissue. Many of the canals are filled with solid masses of epithelium. The stroma is well developed and shows a moderate round-celled infiltration. The larger cystic cavities contain a coagulated secretion. In some places the epithelium presents characteristic papillary growths protruding into the lumena of the canals.*

It is the experience of all cytologists that the finer structures of the tissue cell such as centrosomes, spindle fibres, protoplasmic network and the like, can be satisfactorily demonstrated only when the tissue is fixed at once after removal from the body. By such immediate fixation the parts of the cell are set or coagulated by the fixing fluids before they have much time to change. Such changes are indicated in poorly fixed tissues by shrinkage of different parts of the cell and by the impossibility of demonstrating the various structures. Thus the cytoplasm appears shrunken away from the nucleus; the chromatin in the latter disintegrates while the nuclear membrane appears wrinkled and abnormal. In such cells it is practically impossible to demonstrate complete division figures, the spindle-fibres and centrosomes of the mitotic figure being indistinguishable, while the chromosomes are massed together in formless bulk. Conversely a good test of fixation and of stain is the clear demonstration of each of these parts of a dividing cell. If every spindle fibre is clearly defined, if each chromosome stands out sharply and distinctly, and if the centrosomes appear in their proper places as minute deeply-staining points, while in resting cells, if the nuclear membrane appears full and even, with the accompanying cytoplasm free from vacuoles, then the observer will have material from which he may confidently hope to get results.

It is not easy to get this perfect fixation in epithelial cells, but it is far more difficult to get good results in the structural details of the cell-inclusions. Notwithstanding their unicellular nature even protozoa are difficult to fix properly, as any one can testify who has attempted to fix *Amœba proteus* for example, with its pseudopodia extended, yet it can be done by using proper precautions, and it is equally possible to demonstrate structure in the cancer-cell inclusions. In my opinion the so-called "Plimmer's bodies" or "birdseye" inclusions are but poorly fixed bodies, the structures of which are masked because of poor technical methods.

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*Dr. Gaylord.

Every precaution was used in the treatment of case 300D (cf Figs. 34 and 59). Immediately after removal the tumor was brought in a hot box to the laboratory not 100 feet away, and long before the surgical details in the operating room were concluded, it had been cut into small pieces and distributed among several fixing agents. These included the fluids which have been tested by cytologists and recommended for their power of penetration without distortion. Zenker's fluid, Hermann's fluid, sublimate-acetic, picro-acetic and saturated sublimate were used, the best results being given with the first two. Among the stains used were Heidenhain's iron-hæmatoxylin, Delafield's hæmatoxylin, and Feinberg's stain, all of which gave passable results, but none of these was as satisfactory as the Romanowsky stain of polychrome methylene blue and eosin, nor as the magenta, picric-acid and indigo-carmine stain of Borrel (1901).

A. CRITICAL EXAMINATION OF THE CELL-INCLUSIONS.

The interpretation of a given cell-inclusion in carcinoma can be accurately given only by the genetic history of that inclusion. Hypothesis may follow hypothesis regarding it, but until it is followed out stage by stage the inclusion must remain a puzzle. It is fully as important to get the consecutive steps in the formation of a cell-inclusion as it is to get the series of changes in the life history of some unfamiliar animal, and inclusions in carcinoma will be a source of trouble until this is done for every type found.

Many of the inclusions in carcinoma have an encapsuled appearance due to the presence of a more or less thick membrane separating the inclusion from the cell protoplasm. Others are naked in the protoplasm and, if fixation is imperfect, they are apparently suspended in a vacuole. Secretions, degenerations of portions of the cell, metaplasmic products and the like are naked, and so also are intra-cellular parasites in trophic phases. The great membranes or capsules seen in so many cases are in all probability due to the reactions of the cell protoplasm to foreign bodies and not a portion of such foreign bodies, and the problem of such inclusions may be limited to identification of the contents of the capsule. No such capsules appear in the known cases of intra-cellular parasites, possibly because the protoplasmic reaction which produces the capsule is not called into action, possibly because of some emanation from the parasite which prevents its formation, for it may readily be seen that such a wall would be detrimental to the life-processes of a parasite by shutting off the food supply, walling in waste products and the like. So too the capsule which may form around a foreign body not a parasite; e. g., a blood cell, may cause the disintegration of such a contained cell by similarly keeping out food and keeping in waste products. We may state as a general principle regarding cellular parasites that no living parasite while performing its ordinary vegetative functions within a cell host is separated from its source of food supply by a capsule. A capsule may be formed, however, by the animal parasite itself during its reproductive phases, or the reproductive bodies such as spores may be protected temporarily by such capsules, while after the parasite is dead a wall may be built around it by the cytoplasm of the host cell. This capsule about these carcinoma inclusions then may be used as a convenient morphological element for distinguishing certain histogenetic products within a cell from the metaplasmic products of the cell or from intra-cellular parasites. It may define one type of cell-inclusions, while the absence of a capsule may involve further analysis. If the inclusions are cellular in nature, i. e., provided with nucleus and cytoplasm, they might well be regarded as parasites; if colloidal or uniformly granular, or otherwise specifically characterized, they may be regarded as products of the cell. Obviously, so far as demonstrating the parasitic nature is concerned, the capsule furnishes only negative evidence and the real difficulties in interpretation come in distinguishing products of the cell from non-encapsuled invaders.

Applying these considerations to the inclusions in carcinoma we find that this differential is of value in the interpretation of many of the structures observed. We note the presence of the capsule about cells which, despite their positions within other cells, may be more or less easily identified as leucocytes, or lymphocytes, or other tissue cells which in one way or another have come to be intra-cellular foreign bodies. We also find the capsule about inclusions which are not thus easily recognized, which show no indication of an original blood-cell structure, but by reason of the capsule about them we may conclude that they are derivatives of such invading cells. This conclusion for any specific case is strengthened by reason of more or less definite intermediate stages. On the other hand, there are innumerable inclusions which do not show the capsule and which have an entirely different appearance from the original cell-invaders or their derivatives, and which may be interpreted in some other way. The presence or absence of a capsule thus seems to offer a purely artificial but a convenient way of attacking the manifold structures which have been described again and again as the "cell inclusions in carcinoma."

I. THE ENCAPSULED FORMS.

The thickened rim or capsule about this type of cancer-cell inclusions is a good example of a widely spread protoplasmic reaction. A foreign granule under an oyster's mantle causes local irritation and secretion, resulting in the formation of a pearl. Many insect eggs cause local proliferation of vegetable cells and the formation from those of a thickened coating and other similar phenomena will suggest themselves. While these cases are instances of multicellular reactions to foreign objects, the same kind of reaction may occur when objects are introduced into the protoplasm. A notable example is the thickening of endoplasm about the water in a contractile vacuole in amœba, the condensation resulting in the formation of a capsule similar in nature to the ectoplasm which surrounds the entire animal, and like this brought about by the protoplasmic reaction to the water. It is apparent that the wall of the vacuole in amœba is formed by the compression of the alveoli which make up the structure of the protoplasm of the amœba's body, for the diameter of these alveoli decreases from the endoplasm towards the rim of the vacuole where owing to the density the separate alveoli can no longer be made out.

The conditions of the capsule about a carcinoma cell-inclusion are very similar to those of the thickened protoplasm about the contractile vacuole in amœba. There is the same gradation in density from the very dense inner wall turned towards the inclusion to the outer, normal, cytoplasm of the cell. Ribbert ('91) called attention to the fact that there appears to be a double contour in this membrane and he interpreted it as a thickening in the surrounding protoplasm, but accounted for it by the supposition that when one cell becomes invaginated into another the cell membrane of the invading cell would appear like a capsule. The capsule according to this interpretation therefore would be a part of the invading cell. Other observers interpret the capsule as a condensation of the protoplasm in the invaded cell. This was Sjöbring's ('90) interpretation and it was accepted and confirmed by Sawtschenko ('95), by Ruffer and Walker ('93) and by many others. Sawtschenko observed that the protoplasm of the invaded cell becomes more and more dense as the membrane is approached until in the membrane itself the protoplasm is homogeneous and clearly defined.

The explanation of these capsules is not clearly apparent unless they may be regarded as the result of a protoplasmic reaction to a certain type of stimulus. It is difficult to see how the invagination of one cell by another can give rise to all of the structures provided with capsules in the cancer cells. Ruffer and Walker ('93) express the view that the invaginated cells, due to rapid growth and pressure in a small space, may take the form of a cigarette within its holder. A section of such a combination would show two circles within a circle. In the case of cells, one of which is invaginated within another, we might similarly find an outer membrane of the invaginated cell and two inner membranes, one within the other, belonging to invaginated cell and invading cell respectively, and we could imagine that the nucleus of the invading cell might be pushed to one side as shown in Fig. 20 while some of the nuclear structures might even be interpreted in this way. The difficulty comes, however, in interpreting the degenerated structures within these capsules. If nothing but pressure induces this invasion, what reason is there for the degeneration of the invader and not of the invaded?

The natural supposition would be that it is the latter with its displaced and compressed nucleus in which we should look for ill effects and not in the former. Nor does this theory account for the thickening of the membrane in what may be properly interpreted as inclusions which have been in the invaded cell for some time, nor does it account for the graded density of the plasm which Sawtschenko correctly recognized. While it is not improbable that some of the inclusions found in epithelial cells in carcinoma are invaginations, and that the membranes in such cells may appear like thin capsules, there is reason to believe that this interpretation is too limited to account for all the inclusions with capsules of varying thickness.

Many of the cell-inclusions have no evidence of epithelial cell structure and may be recognized without much difficulty as leucocytes or lymphocytes, etc., and the capsules about them are well-defined and conspicuous (Figs. 2, 3, 5, 19, 20). The possibility of leucocyte invasion is admitted by almost every observer, and it is taken for granted by many that degenerative change of these invaders may give rise to many of the types of cancer-cell inclusions. Sawtschenko ('95) gave a very good review of the possibilities of such changes and described some of the modifications which follow upon invasion. Ribbert attempted to harmonize his theory of invaginating cells with this view by admitting that invagination in some cases might go so far that the invaded cell entirely closes over the invader, and he described the subsequent changes that the imprisoned cell might undergo; most of these, however, apply to the changes which leucocytes or other blood cells undergo and not to invaginating epithelial cells. Klebs ('93) undertook to explain the subsequent changes of invading blood cells on the supposition that they are digested by the epithelial cells which harbor them, and he maintained that many of the structures which other investigators took to be parasites were in reality only stages in the digestion. Without digressing too far to consider this theory, which begs the question of intra-cellular digestion on the part of epithelial cells, it is certainly unquestionable that any cell found within the protoplasm of another cell will, unless it is a parasite and adapted for this particular environment, be unable to continue its own living activities and will die, especially if prevented by a capsule from absorbing food or disposing of waste matters. Kleb's view that the cell is digested seems hardly necessary to account for the disintegration and degeneration which must follow as a natural result from the process of encystment. Whatever the agents are that bring about the degeneration it seems to be a recognized fact that such modifications of leucocytes and lymphocytes take place, and that the products are stored for a longer or shorter time in the protoplasm of the invaded cell.

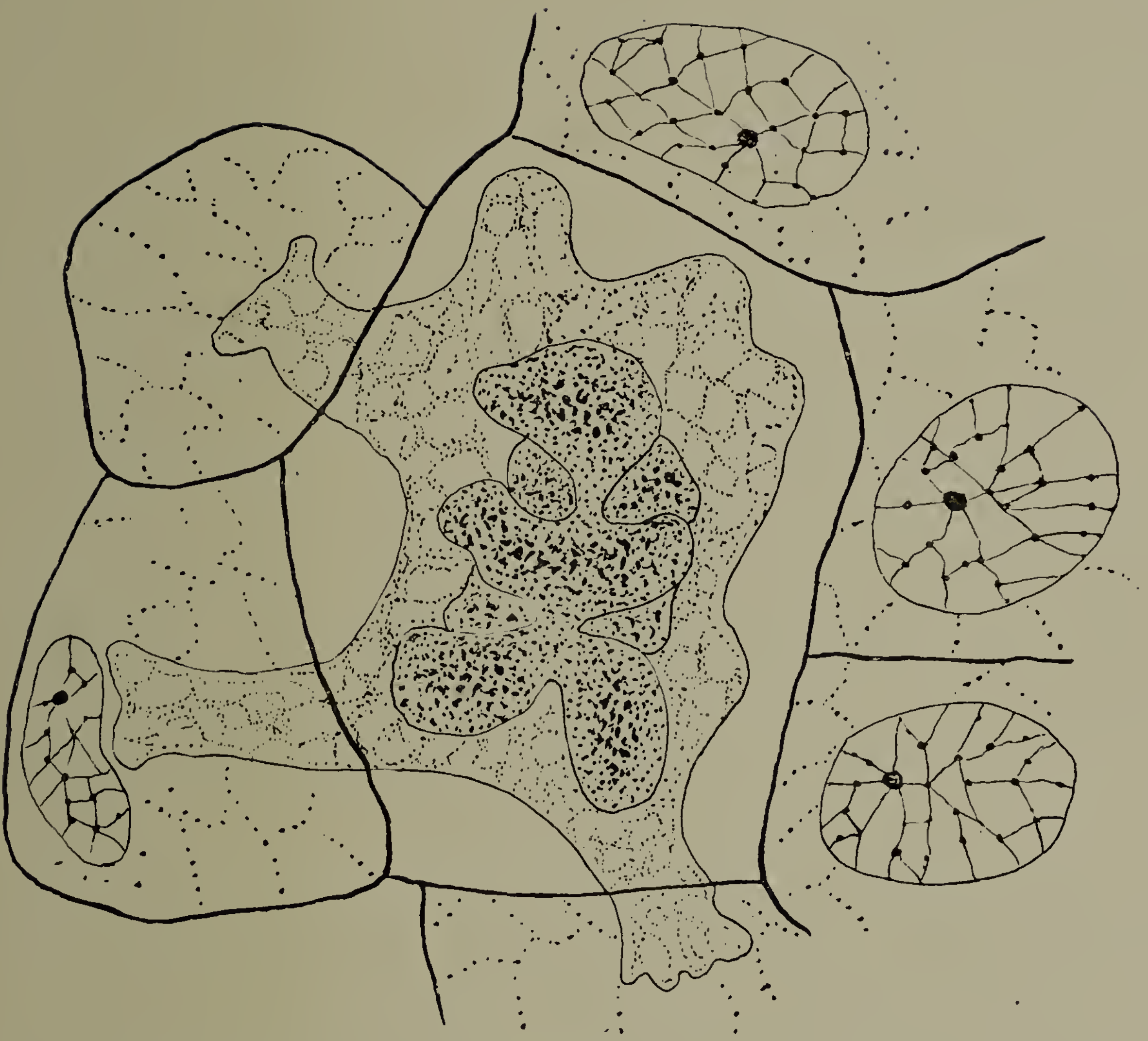
There is little doubt that a structure like that pictured in Figure 2, is a blood cell included in an epithelial cell, and incased within a capsular membrane derived from the protoplasm of the invaded cell. In these cases the original nature of the invading cells is retained and shows the specific character of the inclusion. The capsules are equally characteristic and are very different from the original cell membrane of leucocyte or lymphocyte.

With inclusions of the type represented in Figure 7, the specific nature can not be thus easily recognized and nothing remains except the capsule to aid the identification. This, however, is sufficiently characteristic to warrant the identification as a degenerated blood cell, and the conclusion is strengthened by the study of what are undoubtedly intermediate stages in the disintegration and degeneration of the recognizable blood cells. Here again the capsule plays an important part, for in cases which may be interpreted as later phases of degeneration the capsule appears thicker and denser than in the earlier phases (compare Figures 3 and 6). As Klebs pointed out the first changes which an invading cell undergoes are in the cytoplasm. This becomes "stringy" and incomplete through the appearance of great vacuoles (Figure 19) or it may be drawn out into strands like pseudopodia (Figures 21 and 23) or packed tightly about the yet unchanged nucleus (Figure 2). At a later stage the nucleus becomes modified; its reticular structure disappears, and it becomes compressed into a more or less solid spherical mass of chromatin (Figures 2, 5 and 21). A complete series of these modifications might be obtained without much difficulty from a single cancer, and a few of the characteristic forms assumed are shown in Figures 1 to 7, 16, 17, 19-24, 26 and 38. One important feature is the fact that the cytoplasm of the invading cell does not disappear; that is, it is not digested as Klebs assumed but persists and gives to the body within the capsule an appearance strikingly similar to an amœba, or to a spore of Plasmodiophora within its cyst. The nucleus in such forms varies considerably in size (cf. Figures 21, 22 and 23) and in some cases it apparently breaks up into granules as in Figure 6. Usually it is very much reduced and occasionally appears as a mere fragment of nuclear material within a mass of protoplasm which has no longer even the semblance of a blood cell. Finally this type of inclusion may be traced to structures like those represented by Figures 7 and 24, where hyaline degeneration has resulted in the formation of one or several structureless albuminoid masses with little or no resemblance to the former blood cell.

The earlier writers and observers were inclined to regard these various structures as sporozoa, especially coccidia. This was the interpretation put upon them by Strœbe, by Podwyssotzky and Sawtschenko, by Foa, by Metschnikoff and many others. Some, on the other hand, like Ribbert or Klebs, regarded them as products of degeneration. In addition to these there were still others who were uncertain as to the true significance of these structures, and did not attempt to place them. Among these was Soudakewitch ('92), who declined to place the inclusions which he was unable to identify as degeneration products in any group of animals or plants. Less conservative were Heukolom and Sjöbring, who abandoned the notion of coccidia in favor of the microsporidia, claiming that the inclusions are much more like the organism which causes pèbrine in silkworms than like coccidia. Part of Sjöbring's objection to the so-called coccidia of other observers was correctly based upon the fact that the apparent membrane around the supposed organism increases in thickness, and this he could not harmonize with a capsule formed as a cyst, a structure which, in other sporozoa, when once formed does not increase in thickness.

In reviewing the early work on these cancer-cell inclusions one is struck by the fact that the various degenerating cells of this type have played a most important part in filling in the stages in the "life-histories" of supposed parasites. The majority of observers have described forms of all sizes and shapes varying, as a rule, from 3μ to 40μ, the larger ones with solid and homogenous nuclei being almost invariably regarded as the adult form, and as these are usually found in many stages of disintegration, or as the capsule is frequently found with many structures within it, they were not infrequently interpreted as reproductive bodies ("the sporocysts" of many of the early observers cf. Fig. 7). In one case at least (Korotneff, '92) there appears to have been a confusion of large extra-cellular colloidal masses with these cell degenerations and with the minute bodies which subsequently became known as Plimmer's bodies or the "birdseye" inclusions. Korotneff's supposed organism, which he named *Rhopalocephalus carcinomatosus,* was regarded by him as a connecting link between the coccidia and the gregarines, and was endowed by him with characters of zoological as well as pathological importance. This called out immediate criticism from zoologists and pathologists alike, and the result is that Korotneff's "organism" enjoys the empty honor of a mere historical incident.

Before leaving this type of cancer-cell inclusions we must consider another very questionable organism, which, like Korotneff's Rhopalocephalus, is relegated to the waste pile of zoological genera and species. I refer to the remarkable structures which Eisen interpreted as living, independent amœboid organisms, causing carcinoma. I have reproduced one of these structures from Eisen's paper, and have searched for them in the tissues of the cancer which I have studied. It is difficult to prove that the bizarre cell-structures which I have found are identical with those described by Eisen, but as he maintained that the organisms were very common in cancers of this type I think there is no doubt that I have frequently seen what he means by his *Cancriamœba macroglossa* (compare text-figure and my plate II, Fig. 36). A characteristic feature is the "projectile snout" upon which Eisen bases the specific name, and which he regards as a pseudopodium with the specific function of food-getting. This snout, as he believed, presses into an epithelial cell like the suctorial tentacle of an acinetan and gradually digests the protoplasm of the invaded cell. The nucleus of the parasite is described as polymorphous and is frequently seen in the process of segmentation or fragmentation a process which precedes sporulation.



Copy of one of Eisen's figures of "cancriamoeba macroglossa" to show relative size of cells, "snout" etc.

Without going into details upon the subject of Eisen's interpretation of cancerous growths, and their relations to the supposed parasites, I may give a few criticisms of the supposed amœba, criticisms which are meant seriously since it is quite evident that Eisen's work was done in that spirit. The first point that strikes a student of the protozoa is that the protoplasm of the supposed parasite and that of the epithelial cells is almost identical in structure; the characteristic alveoli of amœba-protoplasm are conspicuously absent. As it is a cytophagous form food vacuoles in which the absorbed protoplasm from the host cells should be digested are always absent; there is no sign of ectoplasmic differentiation, the membrane around the supposed amœba is exactly the same as the membrane of an epithelial cell; contractile vacuoles, while not absolutely necessary for amœbæ, are nevertheless usually present, are entirely wanting in the form under consideration. (Note that in Schaudinn's Leydenia ('96) found in the fluids of peritoneal tumors a contractile vacuole is present.) The nucleus which Eisen fills in with structural detail is entirely different from the nuclei of any species of amœba with which I am familiar, for the characteristic marks of a rhizopod nucleus are large granular masses of chromatin or "chromatin reservoirs" suspended apparently in a nuclear fluid and surrounded by a very definite nuclear membrane. In the cells which I have found in carcinoma and which I regard as the same things that Eisen has described, the nucleus is exactly of the type as those of the surrounding epithelial cells, with the exception that it has lost its definite outline and appears hypertrophied, indented and polymorphic. The chromatin content of the nucleus is very different from that of a rhizopod, for chromatin and limin reticula are readily discernable.

Without attempting to demonstrate the histological significance of this type of structure, which appears to me simply a deformed cell through pressure in the epithelial plug, and undergoing hypertrophic degeneration, I may classify this supposed parasite as another case of mistaken identity which would have been avoided if the author had had a more thorough knowledge of the rhizopod organisms.

Quite a novel interpretation of this type of inclusion was given by Sawtschenko in his later and more important work in 1895. His observations were based upon well hardened and well stained material, and his conclusions regarding the larger cell-inclusions were similar in nature to those of Ribbert and Klebs, who saw in them only the remains of invading cells. Sawtschenko interpreted these inclusions as slimy or colloidal degeneration of cells or parts of cells, but he believed the material so formed to be the food-medium of real parasites of minute size and of varying form. The great majority of inclusions which had been described previously to his work he regarded as merely the scene of activity of the active, living inclusion, which in his earlier paper ('92) he had described as the "tadpole" form. In the later paper these forms were described as present in many different phases and his descriptions are so careful and his drawings so accurate that many of them might well be given to-day as a faithful representation of what is undoubtedly the most important and most interesting type of the cancer-cell inclusions. These inclusions, generally known to-day as Plimmer's bodies, or by von Leyden's term "birdseye" inclusions, have been the subject of contention between the recent advocates of the parasite hypothesis and their opponents. They are the astrospheres and centrosomes of Borrel ('01), the "cancer-parasites" of Bosc ('98), the "Plasmodiophora-like" bodies of Gaylord, the "Histosporidium carcinomatosum" of Feinberg, and the "intra-cellular secretions" of Nósske and Greenough, the Chytridae of Behla as well as the yeast-cell types of San Felice and others; in short, the controversy over the cancer-cell inclusions has been sifted down from the larger structures, which are generally recognized as degeneration products, to these minute inclusions first accurately described by Sawtschenko. The general form of the minute inclusions is that of the familiar Plimmer's body, a vacuole with an inclusion, which with inadequate hardening and staining methods appears homogeneous and usually without structure, thus leading to the misconception of the wall of the vacuole as the cell body and the contents as the nucleus of the supposed parasite. Sawtschenko maintained that the thickened capsule has nothing to do with the parasite save the limitation of the immediate environment, and interpreted the capsule inclusion as cell body plus nucleus. There seems to be little doubt that Sawtschenko confused some stages of degenerating blood cells, especially those forms with thickened capsule, with inclusions like the "birdseye" inclusions which should not be thus interpreted. Many of the cell inclusions of this type were observed by him to have no apparent structure, to be hyaline, and of the nature of colloidal degenerations; in short, the end stages of degeneration, and he suggested that such structures might be the degenerated remains of the parasites themselves and a condition to be expected if the inclusions are parasites, some of which might well be expected to succumb to the reactions of the epithelial cells. This keen observer therefore not only did much to clear the field of the speculative rubbish that had accumulated, but paved the way for future interpretations of the structures which are not to be interpreted as degenerating cells and over which the controversy of a cancer parasite must be fought. Before considering this type of inclusions more carefully it will be well to take up from a general point of view the inclusions which may be grouped together as the non-encapsuled inclusions.

2. NON-ENCAPSULED CELL-INCLUSIONS.

Among the inclusions of this type there are many possibilities. We may include under this heading the various oil or fatty products of the cell, local degenerations in cytoplasm or nucleus, metamorphosing products of nuclear origin, and metaplasmic modifications of various kinds as well as any intra-cellular parasites that may be present. The great difficulty obviously is to distinguish one from the other and any possible parasite from all the rest. On first thought this distinction between a possible parasite and products of the cell itself would appear easy to make on the ground that a parasite must be a complete cell with definite body and definite nucleus, whereas a cell product would be homogeneous and structureless. Unfortunately this is not true. Parasites may be present in which the nucleus is not a clearly defined structure of definite form and size but like the nuclear material of many primitive forms, such as bacteria and flagellates, may be distributed in the form of separated granules. On the other hand, normal structures of the cell may be present which, like the yolk nucleus of many egg cells, or the "Nebenkern" of many early sperm cells, or the "Chromidien" of many protozoa, are nuclear in origin and stain like chromatin, although in the cytoplasm. These are the things in short over which the controversy of the parasite in cancer is being waged and it must be admitted that the advocates of the parasite theory have much to contend with. Some of the inclusions, however, are difficult to interpret except on the basis of foreign organisms, and here we come to the necessity of discriminating between the structures which Behla has aptly termed the X-bodies, and the products of degenerating and invading cells. Let us first examine the other non-encapsuled cell-inclusions such as secretions, degenerations and the like.

A great many forms of cancer-cell inclusions appear homogeneous and without evidence of cellular structure, and might well be explained as degenerations of an albuminous nature. These are sometimes found in the cytoplasm and sometimes in the nuclei of epithelial cells, but wherever seen can be readily distinguished from the other inclusions. The possibility of inclusions being merely colloidal degenerations was pointed out by Soudakewitch in 1892, but the type was not emphasized until more recent times when Sawtschenko, Pianese, Fabre-Domergue, Hansemann, Nösske, Greenough and others have called particular attention to this possibility in connection with the contested inclusions.

It was pointed out by Sawtschenko and Pianese in particular that these colloidal matters may be formed from degenerating portions of the cell or nucleus, and figures have been given by each of these observers indicating the consecutive changes which the structures in question may take. The various *intra-nuclear* parasites of Thoma ('89), of Sjöbring ('90), of Soudakewitch ('92) and Ruffer ('93) were regarded as degeneration products of this type and the formation of chromatin masses of spherical form, vacuolization and changes in color reactions of the masses were described in detail. Such degenerations are common in the cancer which I have studied, and the figures of Sawtschenko and Pianese might have been duplicated many times over. They have also been found in the nuclei of skin cells in smallpox and in diphtheria and similar vacuolization and change in color reaction noted. They are, therefore, inclusions which all students of animal tissues are familiar with, and I may pass them over with the description of one or two. At the outset, as it were, of degeneration, these masses are homogeneous and stain uniformly. (Plate 3, Fig. 39.) Later the central part becomes less sensitive to the chromatin or basic dyes and gradually stains with the acid dyes; the peripheral portion of the mass, however, retains its staining capacity for a longer period and a somewhat shell- or capsule-like structure results. Still later the central portion becomes more disintegrated through vacuolization and may ultimately disappear altogether, leaving a hollow shell or a vacuolated or clathrate structure to mark its place inside the nucleus (Figs. 39-42).

In the cytoplasm similar homogeneous masses may also occur through the degeneration of a portion of the cytoplasm or by the degeneration of an invading cell, or possibly by "intra-cellular secretions." Such changes are known as normal processes in many types of cells which can not be correlated in any way with pathological conditions. Thus in the early egg cells of all types of animals where this subject has been investigated the first appearance of the later yolk material is in the vicinity of the cell-nucleus where it lies like a cap about the nuclear membrane. As the "yolk-nucleus" it has been described by many observers, and in all cases the staining reactions indicate that it is of nuclear origin. With growth of the egg this material becomes scattered throughout the cell body and changes in staining reactions. It ultimately becomes transformed into the comparatively large albuminous "yolk-plates" of the mature egg-cell. In secreting cells of many kinds similar nuclear extravasations have been frequently studied, and the process of chemical metamorphosis has been followed through the many grades of the nucleo-albumens from nucleins on the one hand to clear albumens on the other. In the secreting epithelial gland cells, especially those of the breast, it is to be expected that, if the same origin of the secretion is true, there should be masses of more or less albuminous matter stored in the cell body, and that this matter might show undeniable chromatin staining reactions. It would be expected that such masses might even take a uniform nuclear stain indicating a comparatively high percentage of acid content, or an equally uniform cytoplasmic stain indicating a high and predominating albuminous content, while it is equally to be expected that intermediate grades between the two should be frequently met with. Such inclusions are, indeed, common, and I think there can be little doubt that many of the structures which have been described as sporulating forms of coccidia or large gregarines are to be explained upon this supposition. Even the tadpole forms which Sawtschenko described in his earlier paper ('92) bear the evidences of this mode of origin. This type of structure may not be due entirely to secretions, in the epithelial cells with which we are dealing it may indeed be rare, but similar degeneration may take place in the nuclear material of invading cells which are undergoing disintegration. Thus we may account for structures like those represented in Figure 25, where peculiarly constructed bodies recalling Sawtschenko's "tadpole" forms are distributed about the interior of what appears to be the space occupied by an invading cell in aborted mitosis. This type has been interpreted quite often as a sporulating stage of some organism; e. g., by Soudakewitch, Foa, Metschnikoff, Sjöbring and others, an interpretation which Feinberg, without justification, modifies by considering it the "pan-sporoblast" stage of his microsporidian. In the figure referred to above, some of the contained bodies have a nearly uniform nuclear stain, others a uniform cytoplasmic stain, while in still others part stains with the one, part with the other. The forms assumed by the stained portion are frequently tadpole-like and perfectly homogeneous, and are not sharply demarcated from the remainder of the body. It is to be noted that all of this type of structure are characterized by the fact that the parts which stain with chromatin dyes are on the periphery, and in this respect they simulate the intra-nuclear degenerations described above. Ultimately this type of products of the cell take a uniform cytoplasmic stain and have an absolutely homogeneous appearance.

Still another form of inclusion is frequently found which evidently belongs to this type of colloidal products. These are usually much more fluid than those previously described and give the appearance of oils or fats in the cells. Pianese ('96) has shown the modifications which these inclusions may have, and it is possible to duplicate many of the forms pictured by him in the inclusions observed in case 300D. They are usually inclosed in an irregular area of the cell devoid of capsule-like thickening. The individual globules are of irregular size, probably, as Pianese suggests, due to the fusion of two or more. Figure 27 shows such a type and represents the apparent fusion of a smaller with a larger globule.

3. THE X-BODY.

The so-called X-body of Behla does not find a satisfactory explanation under any of the interpretations of degeneration, secretion, or metamorphosis, and in the cancer literature it has been gradually sifted out from the remaining cell inclusions as a form found only in cancer cells. It is the one type of inclusion which is now generally recognized by friends and enemies of the parasite hypothesis, as the strongest morphological evidence of that theory. It is found in many different phases in the cancer cells of carcinoma, and is the only inclusion in such cells that I have found which in any way justifies the belief on morphological grounds in a possible organism of cancer.

Before giving a review of the previous interpretations of this type of cell-inclusions I would like to describe some of the different forms which it assumes in the cancer which I have been studying. These different forms are so varied and comprise so many different features that at first glance they might be regarded as specifically different things. It is possible, however, to so arrange them that they form a fairly clear and logical set of morphological changes which, if one were so inclined, might be interpreted as growth and reproductive phases of an organism belonging to the unicellular forms. I do not wish to have my meaning misinterpreted in this connection, for there is no positive evidence of a parasite, no sequence in the tissues, and no way to tell, save by possible analogy, that any one stage precedes or follows any other, and to describe in sequence form is partly a matter of convenience, partly to show that such an arrangement is possible, but there is no intention on my part to claim that this sequence in any way establishes the parasitic nature of the inclusions.

In a great many forms of parasitic protozoa the young parasite begins its life as a minute homogeneous spore-like organism with no trace of structure. This is the case in the vaccine organism or young cytoplasmic parasite of the smallpox organism, and the spores of some of the microsporidia which are too small to show the characteristic microspore structures. So in the cancer cells there are minute bodies scarcely a micron in diameter which stain intensely with the specific chromatin dyes (methylene blue, magenta, etc.). These may be the same thing as the so-called Russell's bodies, although I have not observed that they have the characteristic fuchsine reaction which is claimed for these structures and they are much smaller than the forms usually described. They are perfectly homogeneous, sometimes surrounded by a minute vacuole, again with no space about them (Figures 9-10). They are the smallest inclusions that I have seen in cancer cells (less than one micron in diameter), but it is not impossible that still more minute bodies precede these and that more careful technical methods might demonstrate some structure which is certainly not obvious in the preparations as usually made. In slightly larger bodies, equally destitute of membrane or capsule, those for example measuring from 1.5 to 2 microns, there is evidence of differentiation, a minute unstained portion appearing in the body of the inclusion (Figure 10). If we were following the history of an unquestionable organism we should say that the stage following upon the one just described would show a further differentiation of this homogeneous material and that, like a spermatozoon, a portion of the original chromatin-staining substance remains as a nucleus while a peripheral portion forms a more or less clearly defined cytoplasmic or body portion. If this interpretation of these homogeneous bodies were true and if the development proceed as indicated, then the typical Plimmer's bodies might be explained as the immature organism arising from this spore-like germ. It has a definite cell body and a definite cell nucleus, and lies in a characteristic vacuole which always occurs about a foreign body in living protoplasm. In Figure 11 there are two of these forms lying side by side, each is a perfect cell and each is in the form of a minute sphere, measuring 2 and 2.5 microns in diameter. In these particular figures the body of the cell is not drawn out into processes connecting the inclusion with the walls of its vacuole, and this is the usual condition pictured for a typical Plimmer's body (Fig. 8). In the great majority of cases, however, in the preparations which I have studied the inclusion is in connection with the walls of its vacuole by protoplasmic processes which are strikingly like pseudopodia of a myxospore rhizopod-like sporozoon (e. g., Lymphosporidium). The minute bodies are devoid of ectoplasmic modifications and are homogeneous and granular in structure, more dense, however, than the surrounding cytoplasm of the epithelial cell. Occasionally a vacuole is seen in the cytoplasmic portion (Figure 14) but it is not common.

The chromatin-like granule in the center of these small inclusions is perfectly distinct, sharply outlined, and gives an entirely different appearance from the material stained with the chromatin dyes in the types of inclusion described as colloidal degenerations or secretions. It is not a residual color due to the incomplete extraction of the chromatin stain as is the case frequently with colloidal masses or oil-like inclusions, but is a chemically different substance with as definite an outline as a large chromatin granule in the nucleus. This clear definition is retained in all types of the inclusion; sometimes, however, the single granules are replaced by groups of equally distinct granules which may be collected in one group or distributed throughout the small body. With the Romanowsky stain these take the methylene blue, while the minute cell-body takes the eosin; with the Borrel combination the central granule remains red like the chromatin of the epithelial cells, while the cell-body stains green (Figures 12, 14-15, 19, 29, 31, 32, 33, 44-46, 48-51).

The groups of granules are not inconsistent with multiplicative changes in many forms of unicellular organisms. In the rhizopods especially, the nuclear bodies break up into granules by a process called fragmentation; these separate and become the individual nuclei of reproductive bodies. It is therefore a widespread phenomenon of the nucleus and a well-recognized process of division of nuclear material without any of the formalities of mitosis. It sometimes occurs in cases where mitosis is also present, as in *amœba,* where the nuclei divide repeatedly by mitosis until about one hundred are present in the cell, then the chromatin granules in each of the multiple nuclei breaks up spontaneously into small fragments, these escape by rupture of the nuclear membrane, and each fragment forms the nucleus of a spore. These instances serve to show that nuclear fragmentation without a formal process of division is to be expected in the type of organisms to which this cancer cell-inclusion, if it is an organism, may possibly belong. In the small nuclear granule of our form there are no division figures to indicate a mitotic process such as found in the dividing Plasmodiophora for example, but the granules are often seen in an elongated or irregular form, which might be expected in a process of fragmentation. The nucleus-like part of these inclusions is sometimes double, sometimes multiple, but always granular and not homogeneous or diffuse as in colloidal degeneration. The granules are frequently exquisitely minute, so small indeed as to baffle attempt at measurement; the nearest one can get to their size is the general statement that they measure the fraction of a micron, sometimes a large fraction, sometimes small. Whatever the size, however, they are invariably sharply outlined and clearly differentiated. There is never a nuclear membrane, the conditions being similar to the nuclear structures of bacteria or to the protozoon Tetramitus, or Lymphosporidium, or Cytoryctes variolae. Just as in these forms or as in the free non-parasitic types of rhizopods like foraminifera or like the amœba mentioned above the nuclear condition might well justify one in describing this type of inclusion in the cancer cells as a form with its nuclear material in the condition of numerous granular fragments indicating an approaching reproductive process.

It is in this condition of the inclusion, a condition which were we describing a free-living organism might be interpreted as the adult phase, that I have found some of the most interesting and most significant types of the cancer bodies. These are inclusions which give unmistakable evidence of independent protoplasmic movement within the host cells. The most impressive instances of this movement are those cases where the inclusion has been caught as if in the act of migrating from one epithelial cell into another (Figures 13 and 30). In these cases the nuclear material is sometimes in the form of a collection of granules grouped together in a single heap; again the granules are relatively few in number and widely distributed. In Figure 30 the amœboid form is in the act of leaving an intra-vacuolar position in one cell and appears to be penetrating the unaltered protoplasm of an adjacent cell; in Figure 13 a similar condition is pictured, but the connecting strand of material is so thin and delicate that we have the appearance of an indirect division of the inclusion. Indeed this particular case gives the only evidence that I have seen which might be interpreted as a process of division of the inclusion; the chromatin granules are equally distributed and the body is in equal parts. A figure like this, if observed in a free living form like amœba or actinophrys, etc., would be an unquestionable division figure. I have watched the entire process in a minute free form belonging to the genus nuclearia, have seen the pseudopodia drawn in, the appearance of the dividing furrow, the drawing out of the connecting strand of protoplasm and the final break at the center; just prior to the final break the appearance of the two daughter-organisms was strikingly like Figure 13 of the cancer-cell inclusion. It is conceivable that the structure here represented is merely a fragment of a leucocyte and I would be wary of calling it an organism until this possibility is shown to be unfounded.

Still other forms of the inclusions of this type are found in which there is an increase in the number of nuclear granules, each one being separated from all of the rest as in Figures 45, 46, 49 and 50, and occasionally I have found inclusions in which the material is broken up into fragments, each with a chromatin granule in it (Figure 47).

Not only do these inclusions appear in the cell-bodies of the epithelial cells, but they are also occasionally found in the nucleus where they appear to undergo a nuclear metamorphosis similar to that of the cytoplasmic forms. The vacuoles in this case are bounded by chromatin massed to form an apparent membrane which stains with the characteristic nuclear dyes. Several of these intra-nuclear bodies may be traced to metamorphosing chromatin of the nucleus, and there is little or no evidence that they arise by growth of an intra-nuclear spore-like germ. In one case (Figure 28) there are several homogeneous bodies just outside of the nuclear membrane, while two similar bodies are inside of the nucleus and are contained in a vesicle with a delicate wall. Other intra-nuclear forms have the same structure as the majority of the cytoplasmic inclusions, a central or excentric nuclear spot and a differently staining protoplasmic body. In many cases the inclusion appears to be either entering or leaving the nucleus, for the membrane of the latter is deeply indented and the inclusion is partly in and partly outside (Figures 12, 18, 52).

It is quite possible that all of the intra-nuclear forms of the X-body are merely stages in the metamorphosis of the nucleolus as described by Pianese (cf. p. 24). This would explain the chromatin spot and the membrane of chromatin. It might also be argued that the cytoplasmic forms of the X-body are merely the extruded remains of such degenerated nucleoli. The close connection with the nucleus in cases like that pictured in these figures might be cited in support of this theory, but there are so many cases of multiple cytoplasmic bodies, and so many cases where cytoplasmic bodies and nucleoli are both present that the evidence for the theory is outweighed by the evidence against it, while no such theory can account for bodies like those shown in Figures 13, 30, 52, etc.

The large inclusions which had been described up to the time of Sawtschenko, and which have been described by numerous observers since then, were interpreted by him as colloidal degenerations of cells or parts of cells, and a slimy medium was thus formed in which the real cancer-cell parasites found a good feeding ground, and in which they reproduced by the formation of spores of a general sickle-form. The usual inclusions which he described were only once found within the nucleus, the other intra-nuclear bodies which Sjöbring, Soudakewitch and others had laid so much stress on he regarded as degeneration products of the chromatin. His parasites were thought to be more like hæmatozoa than like any other parasitic form and to have a history somewhat similar to the life-stages of the malaria organisms. A nuclear granule was pictured in some forms, a group of such granules in others, while pseudopodial processes formed an irregular peripheral part of the inclusion. The membrane when formed was shown to be a condensation of the surrounding protoplasm of the epithelial cell and no part whatsoever of the organism. It may be seen then that Sawtschenko's observations as to the morphology of the parasite agree very well with the descriptions which I have here given of the

X-body in carcinoma. His material was taken from different kinds of tumors among which were several cases of carcinoma.

There have been many attempts to show that Sawtschenko's "organisms" are products of degeneration, or secretions, or other parts of the normal epithelial cell, but these attempted explanations do not carry conviction, and the failure to find similar things in other diseases, or similarly modified portions of cells in other tissues, goes a long way against such explanation. For the most part these explanations are based upon the possibility of degeneration, or upon some other fanciful change which the cancer cells might mysteriously undergo under the stimulus of unknown toxines or influences of the disease. There is absolutely no doubt that the cancer cells contain inclusions which can be interpreted as degenerations, others as secretions and so on, but in addition to these there are others which can not be disposed of in this way, and these inclusions which Sawtschenko described for the first time, are the forms which remain to be interpreted. An examination of the various attempts to explain them shows the inadequacy of the different interpretations thus far given. Among such attempts we may consider only (1) the astrosphere theory of Borrel; (2) the secretion theory of Nösske and Greenough; (3) the nuclear fragment and chromosome theory of Cornil, Hansemann, Pianese and others.

Borrel's hypothesis has not received much support, nor am I aware that it has been submitted to searching criticism, most observers who deal with the cancer-cell inclusions being content with a simple denial of the possibility. In the early germ cells of the testicles of different animals, especially in many of the vertebrates, the primordial germ cells (spermatogonia) and the maturing germ cells (spermatocytes) have large characteristic structures consisting, as a rule, of several parts, and which are known as the "centrosome" and "sphere" or, all of the structures together, as the "astrosphere." The material of the sphere gives rise in part to the substance of the spindle figure and, when these are present, to the rays passing into the cytoplasm. The centrosomes may in turn have a still smaller internal differentiation the "centriole" and it together with the other sphere substance called the "archoplasm," is supposed to be the center of kinetic activity in the cell. These highly complicated astrospheres are by no means universal in cells and are not easily demonstrable save in germ cells where division is frequent. In epithelial cells they are difficult to make out, although Ballowitz, Heidenheim and Zimmerman have described them in different kinds of epithelium. When present in the cell these spheres are not difficult to stain and are particularly clear and demonstrable when the cells are in active mitosis. At such periods of cellular activity the centrosome and sphere may be seen at the spindle poles, or at the periphery of the daughter nuclei, preparing for the next mitosis. In the resting cells they are conspicuous only in the germ cells or in the spinal ganglion cells of certain animals (dogs, cats, frogs, etc., Rohde). Here they are inclusions with a central spot and an irregular periphery rather sharply marked off from the surrounding cytoplasm but not separated from it by a capsule-like membrane. These astrospheres are the structures which Borrel would compare with the X-body in carcinoma cells, on what justifiable grounds it is difficult to see. Against this view it has been urged that if such structures are normal they would appear in all of the cells while their number would not be increased to twenty or more in any given cell as is sometimes known to happen with the X-body. It seems to me equally plausible to hold the view which Rohde ('03) has recently advanced, that astrospheres in the spinal-ganglia cells are parasites. Both Borrel and Rohde show that the supposed centrosomes divide or else undergo multiple fragmentation to form a group of centers (microcenter) inside of the archoplasmic mass. One (Borrel) interprets the similar division of the central spot of the X-body as equivalent to the fragmentation of the centrosome and regards the central spots as centrosomes. The other (Rohde) holds that the several divisions of the true centrosomes correspond to spores of a parasite. To the former it proves the centrosome nature, to the latter the parasite nature of the central body. To Borrel each part of the cancer-cell inclusion, like the divisions of a centrosome, has the power to form a new centrosome, but each of the divisions of the centrosome forms a new parasite, according to Rohde. To the pathologist the inclusion is a centrosome; to the cytologist, the centrosome is a parasite, and we are justified, I believe, in questioning the advisability of the one on this evidence to enter the field of normal cytology, or of the other to enter the domains of pathology. Borrel's contention can not hold, for the structure in question not only does not have the same history as a centrosome and sphere, but it has no evidence of the structure of this normal part of certain cells, nor is it found at the spindle poles during mitosis, nor at the periphery of the daughter nuclei after mitosis (see Figures 34 and 59). I know of no centrosome or sphere in any normal cell in which there is a like structure, and in cells where astrospheres are formed in large numbers through artificial means, e. g., treatment with magnesium chloride, and which therefore represent abnormal astrospheres of the cell, there is no trace of these peculiar differentiations which are found in the cancer-cell inclusions. In these the artificially created centrosomes and spheres are merely collections of cytoplasmic granules arranged in radiating lines and with a large central granule as centrosome (see Wilson, '02). Borrel's hypothesis then does not stand the test of comparison even with the centrosomes in the spermatocytes of the guinea pig as worked out by Meves, which he takes as his type for comparison, while Rohde's interpretation of centrosomes of this type need not be considered further in this place. It is a gratuitous assumption to hold that the multiple inclusions which are found in many cells in cancer are due to the division of centrosomes, and to explain it we must again draw upon the mystical resources of cancer cells, and to the action of the same unknown toxines that call out degenerations and secretions.

The extremely minute inclusions which have been found in cancer cells and which I have shown in Figures 9 and 10, have been frequently interpreted as minute bits of chromatin or as separated chromosomes. Thus Ruffer and Walker ('93) considered them in this light and agreed with the earlier work of Hauser ('90) in the belief that the so-called Russell's bodies are nothing but portions of the nuclear material which have been derived in one way or another. That there is some good basis for this assumption is indicated by the fact that chromosomes are frequently known to get separated from the nuclei in which they belong and to lead an independent existence in the cell protoplasm. Thus Juel showed that a separated chromosome forms a nucleus of minute size in the cell; it loses its homogeneous appearance in the same way that the chromosomes within the daughter nuclei do, and Juel showed that it forms a reticulate nucleus with chromatin network of the same type as the larger nuclei. Its ultimate fate was not followed. Similar isolated chromosomes have been studied by Boveri and others, and the possibility of such an origin here is not excluded.

Again, the nucleus frequently appears wrinkled and warty and minute protuberances which appear to be on the point of detachment are frequently observed; these are often parts of leucocyte nuclei, the structures par excellence for fragmentation. Figures 57 and 58 show the possibility of such an origin of certain of the minute forms in the cancer cells, and possibly other minute structures, which I can not interpret, may have such an origin (e. g., Figure 37).

Without denying the possibility of such an origin for many of the X-bodies, I do not think that the explanation covers all of them by any means. The origin from a chromosome or from a portion of the resting nucleus might explain the nuclear part of the inclusion, but it fails to indicate the origin of the cytoplasmic part, or the nuclear granulations and the general cell-like structure of the minute inclusions, or of the motile forms as shown in Figures 13 and 30. The inclusions are not like nuclei as described by Juel, but are perfect little cells, each with its minute granular nucleus and alveolar cytoplasm.

Several observers have attributed the X-bodies to secretions in the cell. Pianese, Hansemann, Greenough and Nösske all have a more or less definite conception of the process by which the secreted matter may become hardened or more dense and so give rise to chromatin-like portions. Greenough refers them to specifically modified protoplasm belonging possibly to the cellular structures which Prenant described, and according to whom the protoplasm of a tissue cell contains many kinds of "higher" protoplasm, quite unintelligible to the ordinary cytologist. Nösske interprets the

X-bodies as nothing but vacuoles in the cell which become filled with some kind of inner secretion which is capable of a coagulation process, through which the nucleus-like granules are obtained. In all of these cases there is the same uncertainty of the *modus operandi* in the formation of the questionable body; in each case there appears to be an *a priori* conception of how it might take place, but we search in vain for morphological light on the process. In the meantime the structures are there, definite, clearly differentiated, and of practically the same type, so that they are unmistakable. According to this theory their presence in the nucleus involves that organ of the cell in a new rôle, viz: a "secretion-receptacle," while the unmistakable signs of movement on the part of the inclusions will necessitate a new manifestation of mental agility on the part of the secretionists. My own experience does not enable me to picture secretions in these manifold rôles and in the varied positions, and until more positive evidence is adduced than the literature affords at present, the balance of the evidence must go against this interpretation also.

The X-body thus is not satisfactorily explained as a nuclear product, nor as an astrosphere or secretion. Can it be referred to degeneration products of invading blood cells? The form of the minute inclusion is similar to that of the stage in degeneration where the nucleus is compacted into a homogeneous sphere with cytoplasm in the form of pseudopodia, while the multinucleate condition is duplicated by fragmentation of the nucleus of the degenerated leucocyte. Late stages of the degenerating blood cell may be strikingly like some inclusions which might be mistaken for stages of the X-body. Thus Figure 16 and Figure 17 are very similar, and despite the absence in the latter of the enveloping capsule may be identical in origin. The inclusion shown in Figure 17 is apparently free and resting on two cells, and as it appears under the microscope it is not an intra cytoplasmic structure. This may account for the absence of capsule like that of Figures 16, 23, 38, etc. These are very different things from the more minute and delicate structures shown in Figures 12, 15, 18, 19, 31, 32, 44, etc., and I do not see how the latter can be referred to degenerating blood cells unless, indeed, they are mere fragments of leucocytes; they have no capsules, they are provided with definite chromatin granules and have a well-defined protoplasmic structure. It is an easy way out of a difficulty to say that such things are derived from degenerating tissue elements, and such statements too often given as mere dicta, can not advance the subject in any way.

It is equally unscientific to maintain on the evidence thus far obtained, that these things are parasites. As a working hypothesis, however, it is perfectly legitimate to interpret the various forms of the X-body as organisms quite different from any products of histogenesis. Thus Figures 13 and 30 represent a type of these cell-inclusions which the trained microscopist can not pass over lightly as degeneration products, secretions, or other ordinary cell derivatives. The definite contours, the brightly staining points of chromatin, the evidences of spontaneous movement, all tend to strengthen the hypothesis of a foreign intra-cellular organism. It is conceivable that the structure represented in Figure 13 has been drawn out by division of the cell, and that the connecting strand was not severed after complete separation of the daughter cells, but this seems hardly possible when we consider the age of these cells as shown by the nuclei and cell membranes. The cell plate forming between the daughter cells would certainly have ruptured the delicate connecting strand of the inclusion. The vacuole at the lower end, and the absence of the vacuole at the upper end indicate movement towards the latter.

Notwithstanding the many significant forms assumed by the X-body, I do not yet share the certainty of those who have felt sure enough of the parasite to give it a specific name and a specific systematic position in the animal or vegetable series. The wrecks of *Rhopalocephalus carcinomatosus,* of *Cancriamœba macroglossa,* of Sjöbring's *Strombodes,* indicate the dangers of such attempts, and Feinberg certainly takes great risks in giving to the X-body the specific name of *Histosporidium carcinomatosum.* Even though the X-body were an undoubted organism, which, by the way, is not evidenced in any degree by Feinberg's descriptions, there is absolutely no structural or ontogenetic evidence to justify its position as a sporozoon. There is not enough structural evidence even to place it definitely as plant or as animal and such attempts are premature. If on the evidence thus far advanced we were inclined to indicate relationship of the possible parasite, we would, with Behla, place it with the Chytridiaceæ, or with some other similar group intermediate between the lower plants and protozoa. Even were it proved to be an organism it could be accurately placed only after knowledge of the full life history, and the evidence even for simple reproduction by spore-formation, the possibility of which is barely indicated by structures similar to that represented by Figure 47, is not yet forthcoming. The difficulties of the problem make it the more fascinating, and the parasite hypothesis should not be deprecated or neglected because of the hasty conclusions which some of the advocates of that hypothesis have published. I am much more favorably impressed with the breadth of view of Borrel which prompts him to say: " The criticism which I have just made against the attempts to explain these inclusions as sporozoa should not prevent further research along the lines of this seductive hypothesis " (op. cit. '01, page 60), than with the dogmatism of Marchand when he says: " Since the theoretical considerations are totally opposed to the probability, and indeed the possibility, of a parasitic origin of carcinoma and other malignant neoplasms, and since all cell-inclusions which have been described as parasites are not such but are products of the cell itself, it is high time that pointless research for a carcinoma parasite should be given up and the cause of the malignant growth sought for in other ways."* Even though a specific parasite in carcinoma on *a priori* grounds seems untenable to some, the possibility that such is the case is at least conceivable, and this line of attack should not be neglected until the ætiology of cancer is established. But the *a priori* arguments which have been given are not sufficient to show that this theory is untenable, and the opponents of the theory do not take into consideration all of the possibilities of activity of living foreign inclusions.

Protozoa, like the bacteria, produce substances as products of their metabolic activity which are poisonous to their hosts, and give rise to malignant diseases. Malaria, smallpox, scarlet fever and the several types of disease caused by different species of the genus Trypanosoma owe their morbid effects to toxines of this cellular origin, and these affect the host in various ways, sometimes only physiologically, sometimes both physiologically and morphologically. The specific effect produced in carcinoma is the increased activity of cell division in epithelial cells leading to proliferation of these cells, to abnormal structures and to invasion along the lines of least resistance. The usual response of a lower animal to the intercellular protozoon parasite is the formation of an enveloping sheath or cyst due to accumulation of the connective tissue and called out by stimulus of the parasite. In some cases, however, there is a stimulus to division of the epithelial cells and unusual proliferation in the vicinity of the infection. Thus Thelohan, Hofer and Doflein emphasize the fact that Myxobolus-infection in fish results in the unusual proliferation of the muscle-cells; Leuckart called attention to the epithelial proliferation in Coccidia-infected rabbits, an observation which Tyzzer ('02) has recently confirmed. In vegetable protoplasms the effect of *Plasmodiophora brassicæ* upon plant tissue is the active proliferation of cells and the formation of great deformities in the roots. Proliferation therefore is a general phenomenon attendant on the presence of protozoon parasites.

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*Quoted from abstract in Centralbl. of Bakt. Par. 1903.

The objection has been raised that the supposed parasites in carcinoma are so few in number that they are totally inadequate to account for the active increase of the epithelium, and as a counter-proposition it has been urged, especially by Cohnheim, that the invading epithelium is a reserve of embryonic cells which have lain quiet in the body until the proper conditions bring out the latent energy and cause them to rapidly divide like young cells. I am not competent to discuss this theory, nor to criticize it from the embryological point of view, and for this reason the fact that it seems to me farfetched and improbable, will carry little weight. I am more at home in criticizing the recent view of Ribbert ('01), who makes the objection that if parasites are the cause of cancer they must have the very novel power of changing the entire nature of epithelial cells; that is, of generalizing them and of making them more perfect cells instead of more disabled cells, or that the parasites instead of exerting a harmful effect upon the cells they infest produce a beneficial effect. A satisfactory response to this kind of argument was made by R. Hertwig (1900), who called attention to the fact that cancer cells instead of being perfect or embryonic in nature, are actually the reverse and show unmistakable signs of senile degeneration. He called attention to the fact that in protozoa (Actinosphærium) after a long period of rapid asexual reproduction the cells become hypertrophied and abnormal in structure; the nuclei are much enlarged and show characteristic morphological changes which are due to protoplasmic senility. The fact that such changes are frequent in cultures of protozoa which have been kept from sexual reproduction for long periods has been known for many years, and old age in these single cells is a well-known phenomenon since Maupas' classical researches in 1888 and 1889. Hertwig's contention is that abnormal epithelial growths do not occur in the young human tissues, but make their appearance after cell-proliferation has become less active. In such cancer cells, the abnormal growth is a sign, not of youth with its well-balanced self-regulation which prohibits abnormalities, but of senility in which the self-regulating power so characteristic of all living things has been lost. Secondary evidences of this degeneration are shown by the hypertrophied nucleus, frequent amitoses and loss of the specific characteristics of the tissue involved. The problem of the ætiology of cancer deals with the direct cause of this special increase of the dividing energy of cancer cells.

An epithelial cell in an adult human being represents the Nth generation of cells since the first cleavage of the egg, and just as we are justified in saying that the 319th generation of Stylonychia pustulata, or the 200th generation of Paramœcium caudatum represent the limits of dividing energy of these two organisms, so we may say that the Nth generation of any tissue cells represents the limit of dividing energy in these human cells. In the case of Paramœcium, however, it has been shown that the waning energy may be renewed by artificial means, and that the addition of a little salt (potassium phosphate) to the medium in which they live for the short period of thirty minutes, was sufficient to stimulate the worn-out cells to a renewed activity, and instead of dying like the organisms not thus treated they divided about 200 times more. This process was repeated again and again until the race finally succumbed in the 742d generation (see Calkins '04). The dividing power of the worn-out cells of an epithelium is capable of similar reinvigoration, even after the limits of growth have been attained, and renewed proliferation may take place in functional epithelium, giving rise to abnormal growths which have been explained without calling upon improbable and inexplicable embryonic tissues.

The stimulus to the renewal of the dividing activity of an epithelial cell in the case of carcinoma may very well come from the inclusions, notwithstanding the relatively small numbers present. Owing to the small size of these inclusions I am inclined to think that they are frequently overlooked, and that their scarcity is somewhat traditional. I have found them in abundance in a rapidly growing carcinoma where they are supposed to be less numerous than in the older, more slowly-growing types. If the analogy with the worn-out race of Paramœcium holds good for the epithelial cells, then would it be unnecessary to have a parasite for each cell as Ribbert assumes; a single stimulated cell, if reacting like Paramœcium, would divide again and again after stimulation, and the effects of the parasite would be a Fernwirkung in a new sense.

In a certain sense the stimulus which a parasite might thus produce is not unlike the action of a spermatozoon in the egg. In relative volume to the egg cell the male cell is no greater than the relative volume of the X-body to the epithelial cell, and yet it produces the wonderful result of development. Its action has been interpreted as an effect of enzymes, and in support of this explanation the work of Loeb and others on artificial parthenogenesis has been cited. The same effect as that of a spermatozoon has been produced by a minute quantity of magnesium chloride, and the stimulus has thus been given for the egg to divide again and again, while self-regulation and differentiation work together to mold the cell progeny into the form and structure of the organism. Here then is the temporary action of a foreign substance upon living protoplasm, the effects of which are felt long after the direct contact, and another instance of this special type of **Fernwirkung.**

If a toxine of this type is at work in carcinoma it would not be unreasonable to assume that a certain optimum is necessary to produce the stimulating effect; if this optimum is exceeded, growth may be retarded; if the proper amount is not present the effects will be nil. In the experiments with Paramœcium I found that a certain quantity of salt and a certain time of its action were necessary for successful results, and the same experience has been met with by all who have attempted to induce artificial parthenogenesis in eggs. This may be one explanation of the slow growth of cancers in which the number of the X-bodies in the epithelial cells is relatively large. Upon such an hypothesis it may be seen that while possible carcinoma parasites have no such effect as the smallpox organisms for example, such parasites might be quite sufficient to stimulate cell development and tumor formation while the ensuing morbidity and ultimate cachexia might well be traced to general poisoning due to a disordered system and not directly referable to the parasites themselves.

SUMMARY.

1. There are two main groups of inclusions in carcinoma, those which are encapsuled, and those without capsules.

2. Encapsuled inclusions appear under several forms, sometimes definitely cellular in which case they are recognizable as blood cells or epithelial cells, sometimes variously modified by degenerative changes and unrecognizable.

3. The various modifications of such inclusions have been variously and erroneously interpreted as stages in the life history of protozoan parasites.

4. Attention is directed to the fact that parasites in cell-protoplasm can not get food nor rid themselves of waste products when enclosed in capsules.

5. The same principle may account for the degeneration of encapsuled invading cells such as leucocytes, lymphocytes, etc.

6. Capsules or cysts around parasites are formed not by the host cells but by the parasites at periods of reproduction or for protection

of spores, etc. Capsules around cell inclusions in carcinoma are formed by the protoplasm of the invaded cells.

7. Non-encapsuled inclusions in the form of secretions, degenerations, metaplasmic modifications, etc., are likewise present in cancer cells. These must be correctly interpreted before any parasite hypothesis is advanced.

8. The X-body of Behla is not satisfactorily explained as metaplasmic, degeneration or secretion product, although it is not yet known to be independent from blood-cell origin. It appears without a capsule but lies in a vacuole in the cytoplasm or nucleus. It possesses a granule or granules which take up the chromatin dyes with marked intensity. In some cases it shows strong evidence of spontaneous movement.

9. Theoretical considerations are not entirely sufficient to outweigh the morphological evidences of an organism. The small number of inclusions indicates that no such results are to be expected as those which follow the presence of masses of parasites as in smallpox or scarlet fever, or malaria, but experimental results show that great proliferating stimuli may be given by minute quantities of poison, and *a priori,* grounds are thus obtained to justify the effect, in this case a tumor, through the activity of a relatively few parasites.

10. The proliferating epithelial cells of carcinoma do not show the characteristic of embryonic cells, but rather of senile degenerate cells as shown by cell and nuclear hypertrophy, and by loss of the self-regulating power which is particularly characteristic of young or embryonic cells.

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Description of Plates.

All figures are drawn with the camera with lens system of ocular No. 6 Zeiss and Objective 1.5 Zeiss. Magnification about 1,800 diam. All inclusions represented are from one case of carcinoma.

Plate I.

Fig. 1. A cell-inclusion of unknown origin but probably a degenerating epithelial cell.

Fig. 2. Epithelial cell with invaded blood cell (lymphocyte).

Fig. 3. Cytoplasmic degeneration beginning in invading leucocyte. The nucleus of the invader is not yet affected.

Fig. 4. Degenerating lymph cell in which no trace of cytoplasm remains.

Fig. 5. Further stage in the degeneration of blood cell. The chromatin of the nucleus is massed, although not so densely as in the preceding figure.

Fig. 6. Late stage of nuclear metamorphosis of an invading blood cell. The thickness of the capsular rim is characteristic of the inclusions in this stage.

Fig. 7. Colloidal masses in a vacuole with capsule, representing possibly, the end phase of degeneration of an invading cell.

Fig. 8. A "Plimmer's body" consisting of vacuole, central spot and rim. Indicating no differentiated granules like those of the X-body.

Fig. 9. Homogeneous spot of foreign substance in cytoplasm, characteristic of many inclusions in the epithelial cells.

Fig. 10. Similar body somewhat larger, with a characteristic vacuole.

Fig. 11. Two "Plimmer's bodies" with central, differentiated spot.

Fig. 12. The X-body of Behla, indenting the nuclear membrane, and with highly characteristic form including pseudopodial processes which may be traced to the periphery of the vacuole, and an internal differently staining spot which is always sharp and clearly defined.

Fig. 13. A form of the X-body which was seen only two or three times. It stretches between two adjacent cells and each enlarged end contains three distinct spots which take the chromatin dyes.

Figs. 14 and 15. Two characteristic X-bodies.

Figs. 16 and 17. Two similar amœboid masses with definite granular masses of chromatin-like substance. One has a capsule, the other has none. These cannot be organisms but their origin is not made out.

Fig. 18. An X-body partly embedded in the polymorphic nucleus of an epithelial cell.

Plate II.

Fig. 19. Epithelial cell with leucocyte invader, and minute X-body of characteristic form and content.

Figs. 20 and 21. Two stages in the degeneration of an inclosed blood cell. In the latter the nucleus is becoming homogeneous and contracted through the loss of nuclear fluids.

Figs. 22 and 23. Two later stages in the degeneration of invading blood cells. The compact nucleus is much reduced in size and the cytoplasm appears broken.

Fig. 24. Last remains of what is apparently the end stage in degeneration of the inclosed cell; nothing is present but a homogeneous central dot, while the remainder of the cavity inside the capsule is flocculent and unformed.

Fig. 25. A figure representing a late stage in the degeneration of a cell with chromosomes. These are the "tadpole" forms described by Sawtschenko and interpreted as parasites in the slime formed by a degenerating cell.

Fig. 26. A cell with nucleus broken into many fragments which appear uniform in size and characteristic enough to be regarded by many as stages in the reproduction of a parasite.

Fig. 27. Fat-like particles in an epithelial cell showing fusion of droplets.

Fig. 28. Nucleus of epithelial cell with several questionable bodies in the cytoplasm and one similar body in the nucleus. Suggested as a possible early phase of the intra-nuclear X-body.

Figs. 29-33. Various phases of the X-body. In the last two the chromatin material is in the form of minute granules resembling the nuclear conditions of many lower forms. In figure 30 the X-body has the form of an amœboid organism apparently in the process of migrating from the vacuole into an adjacent epithelial cell. It is very difficult to interpret this form as a degeneration product or portion of a leucocyte. The chromatin material is in four separated granules, one at each end of a pseudopodia-like process.

Fig. 34. A stage in the anaphase of mitosis showing chromosomes and spindle fibres but with no trace of the X-body at the poles as would be expected were Borrel's centrosome-hypothesis correct.

Fig. 35. An intra-nuclear X-body with typical structure.

Fig. 36. An enlarged epithelial cell with hypertrophied nucleus and curious neck-like process; a type of cell which was supposed by Eisen to be a specific parasite of cancer and named by him "Cancriamœba macroglossa."

Fig. 37. A cell with a minute inclusion of unknown origin. The appearance of the minute elements within the vacuole suggests that it is derived from a misplaced chromosome, but not enough stages were observed to warrant this interpretation.

Plate III.

Fig. 38. A stage which is difficult to interpret without comparison with the earlier and similar figures. It represents a rather late stage in the degeneration of an invading cell with the compact nucleus undergoing secondary vacuolization preparatory to the formation of numerous granules as represented in figure 6.

Figs. 39-42. Stages in the metamorphosis of the "nucleolus" of epithelial cells, showing the hollow sphere, the reticulated nucleolar body (figure 40, compare with this figures 53 and 54) the clathrate body and the degenerate vesicular body with change in color reaction.

Fig. 43. A stage analogous to that of figure 24.

Figs. 44, 45, 46. Various stages to show the nature of the chromatin in the X-body in the form of separated granules, sometimes of small size, sometimes large.

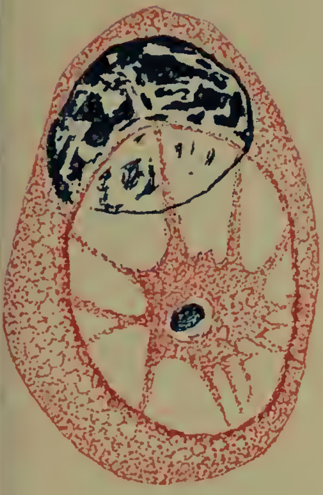
Fig. 47. An interesting stage suggestive of reproductive process in some single-celled organisms. Each chromatin granule has its separate bit of cytoplasm.

Figs. 48-52. Similar types frequently found in this cancer.

Figs. 53-56. Characteristic intra-nuclear forms which are identical in structure to the X-bodies in the cytoplasm. Possibly to be identified as degenerations of the nucleolus as shown in figure 40. Figure 56 is a curious structure which were it not isolated, might suggest a stage in merozoite-formation of a coccidium, but is probably only a particular type of nuclear degeneration.

Figs. 57 and 58. Two nuclei with buds showing the possible mode of origin of structures like those shown in figure 10, etc.

Fig. 59. Another mitotic figure in the metaphase stage showing that the spindle-poles, although provided with centrosomes have no trace of astrospheres.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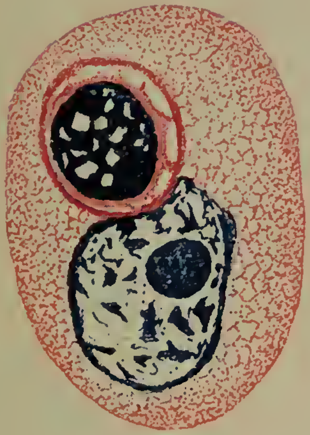
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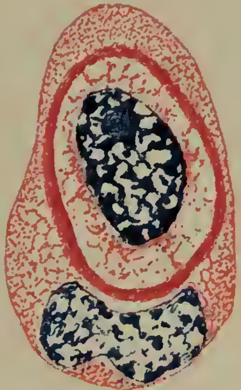
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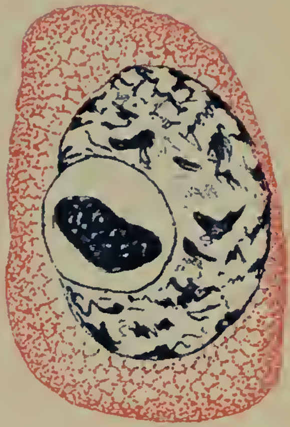
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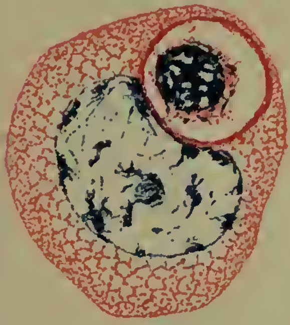
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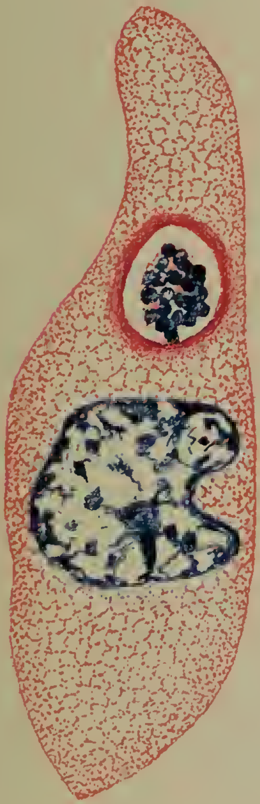
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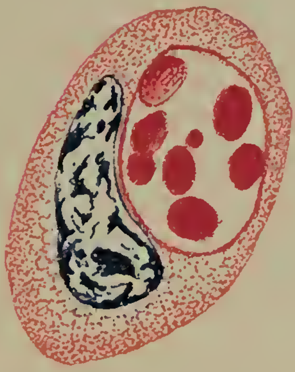
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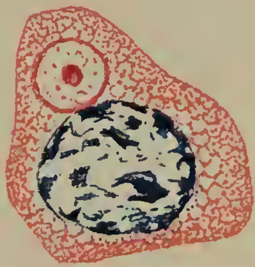
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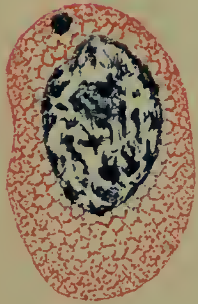
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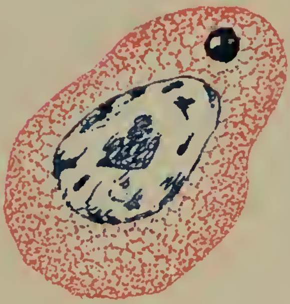
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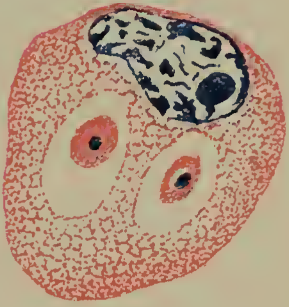
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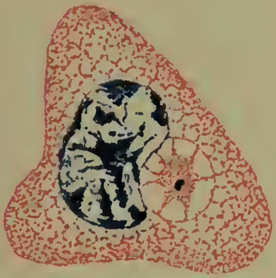
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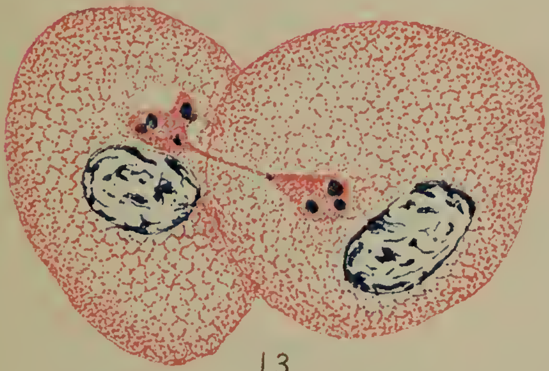
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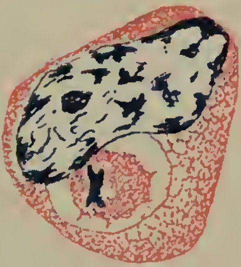
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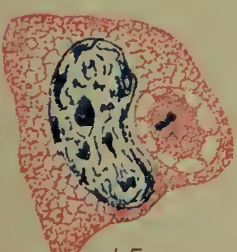
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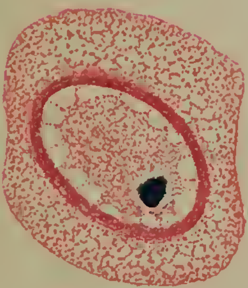
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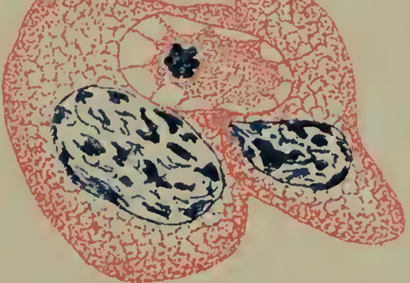
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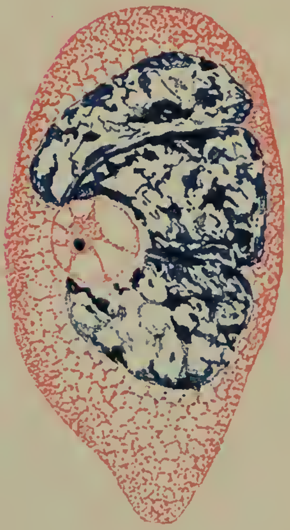
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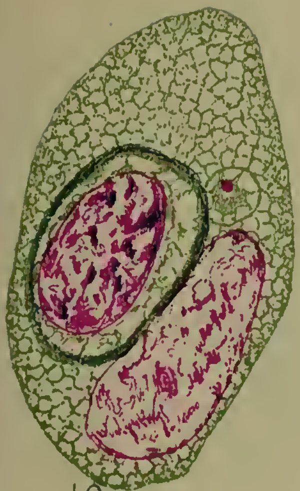
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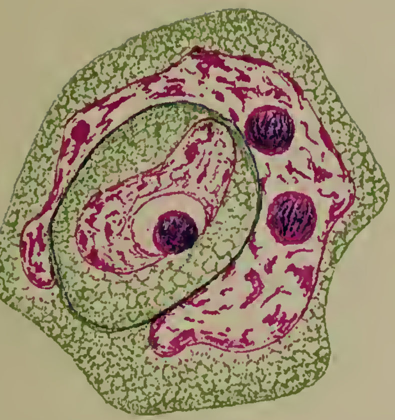
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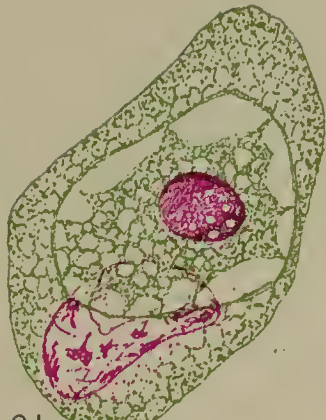
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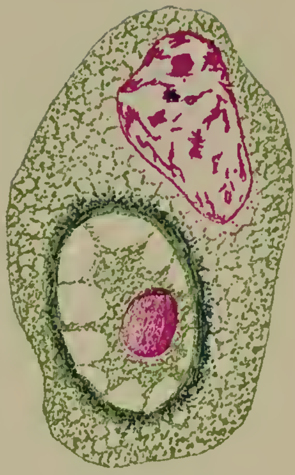
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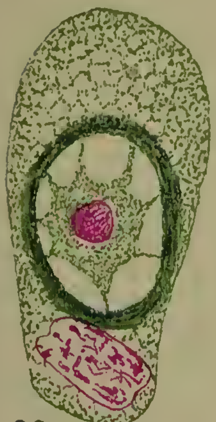
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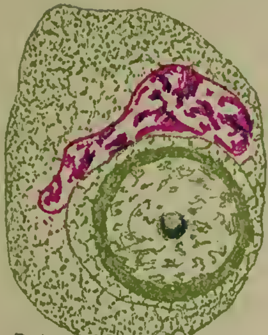
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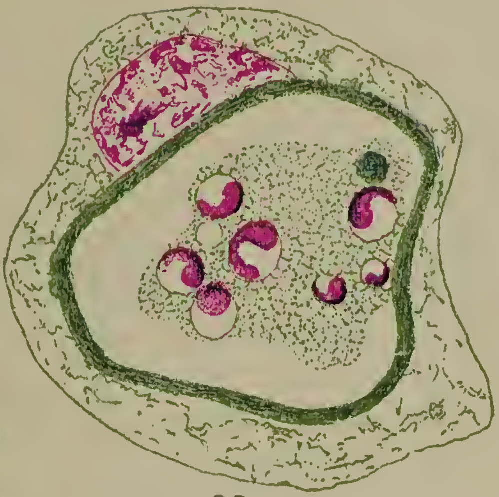
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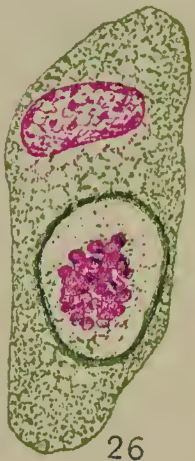
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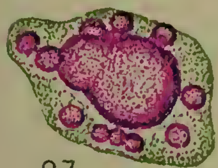
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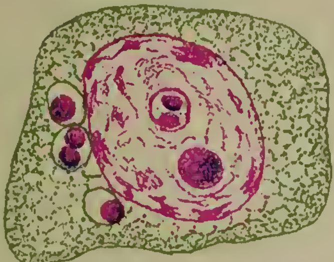
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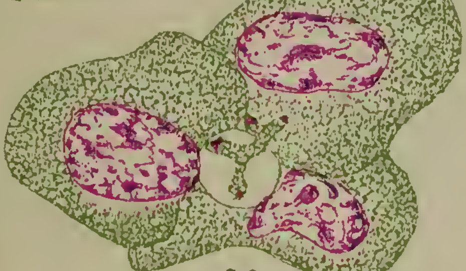
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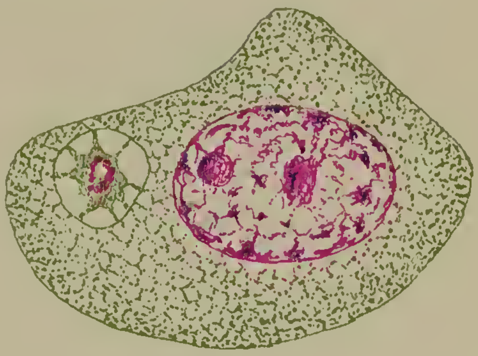
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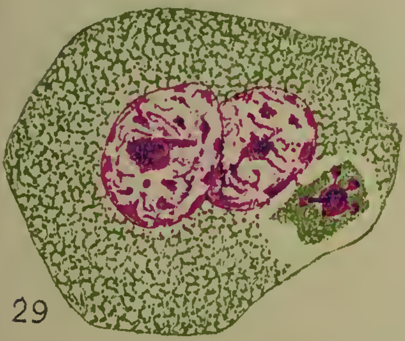
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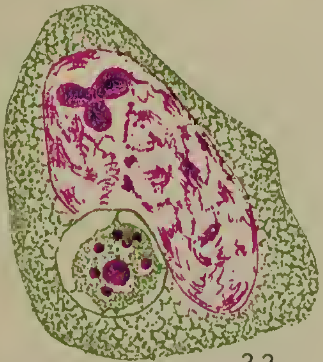
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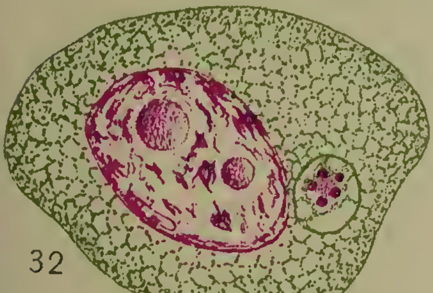
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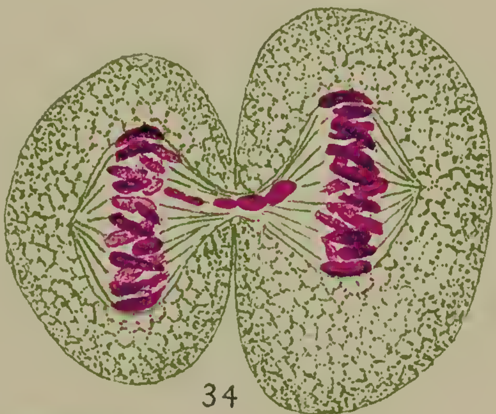
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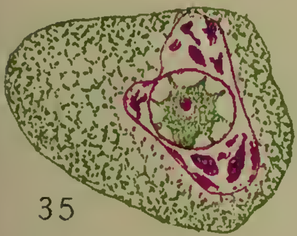
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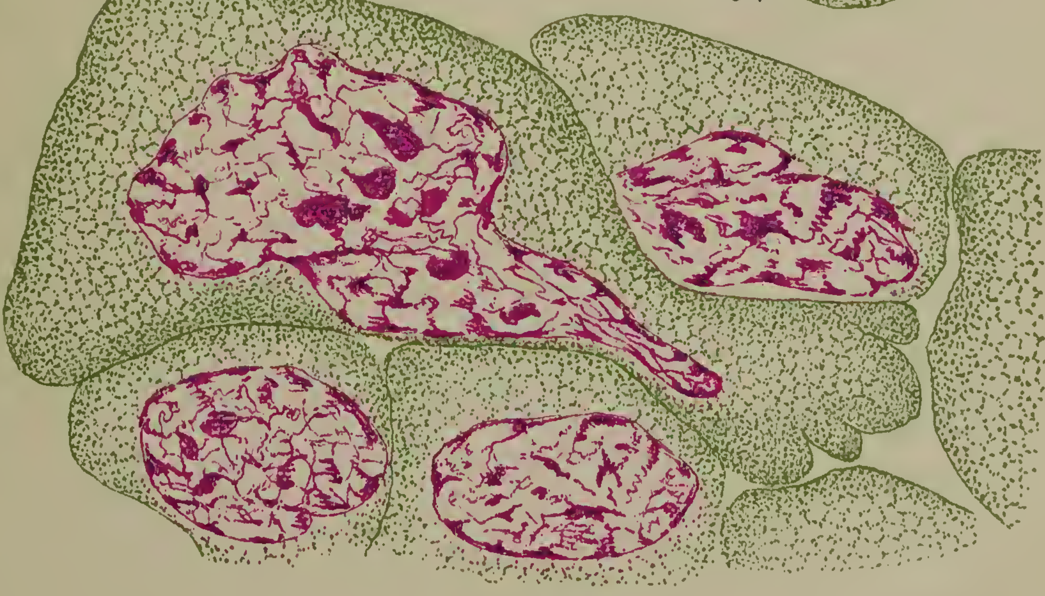
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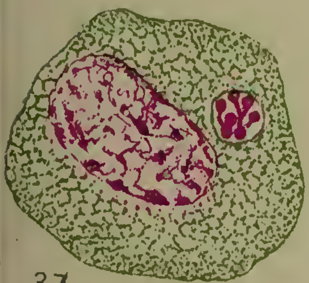
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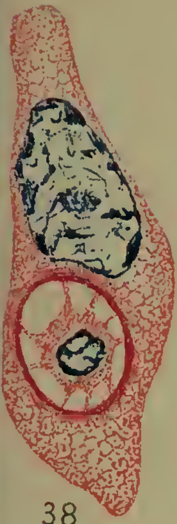
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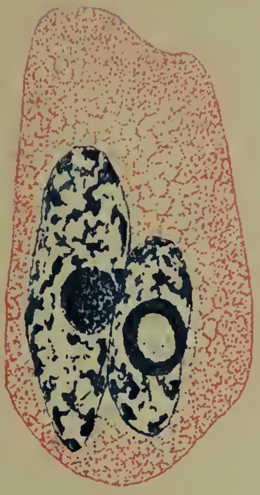
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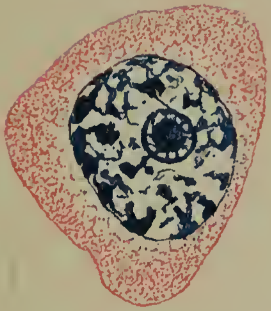
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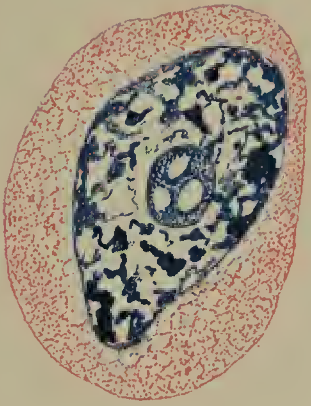
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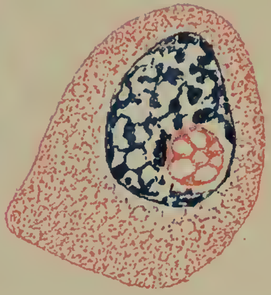
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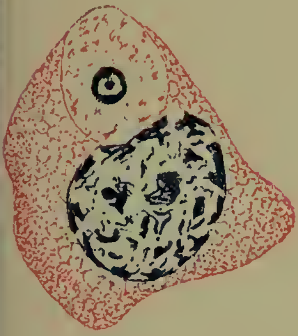
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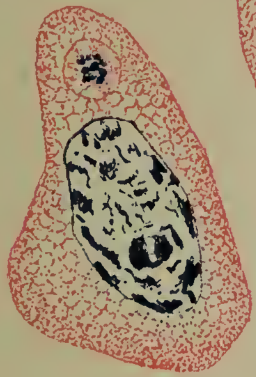
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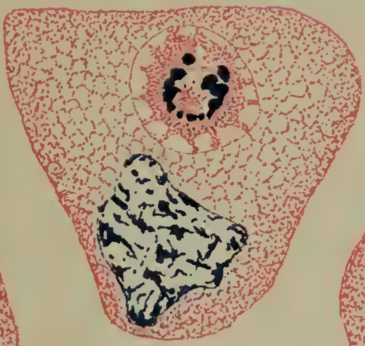
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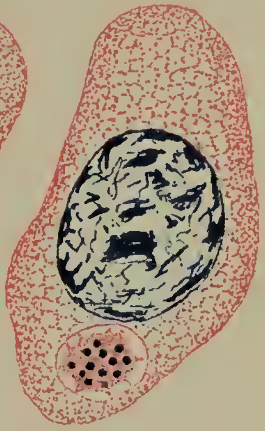
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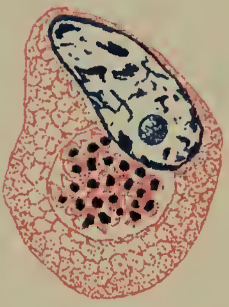
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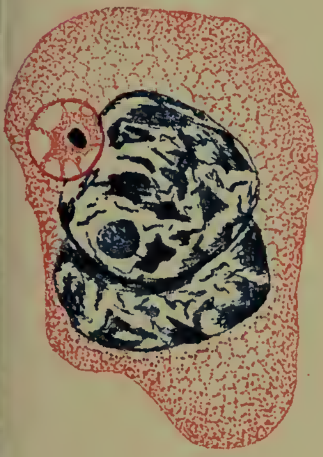
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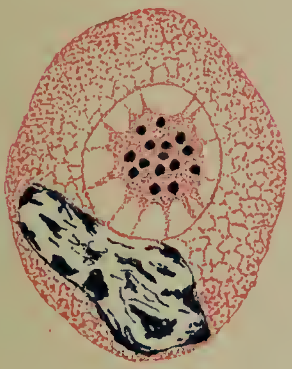
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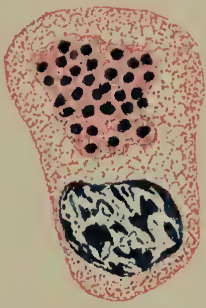
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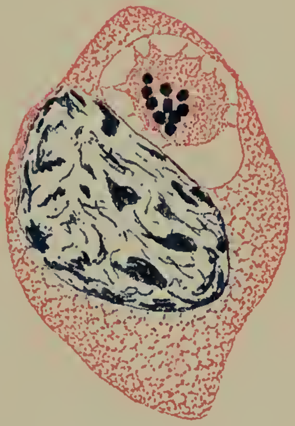
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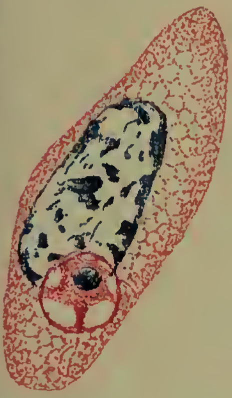
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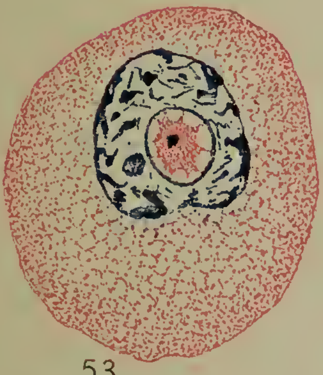
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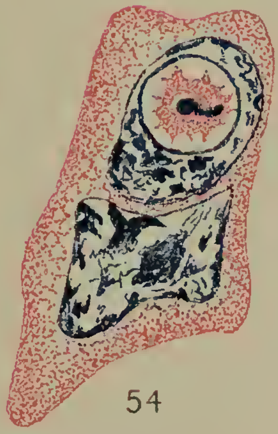
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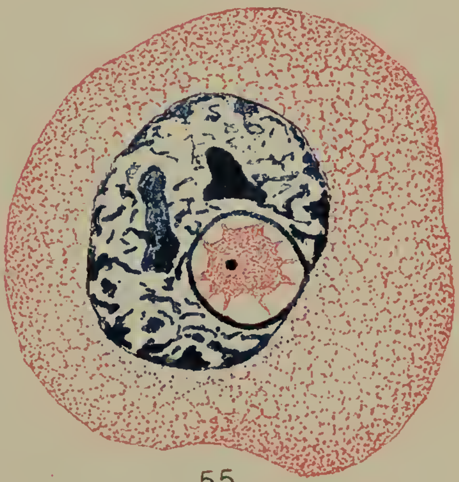
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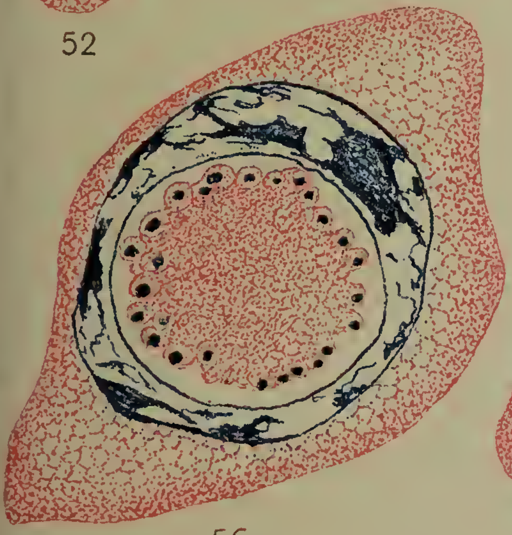
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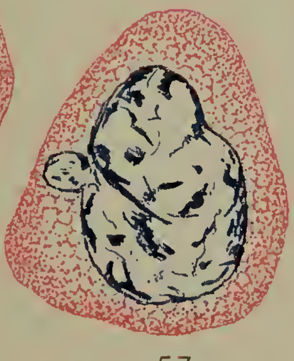
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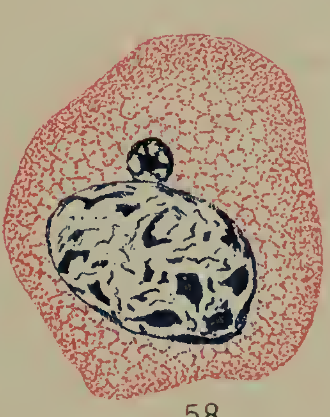
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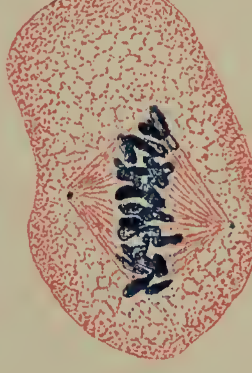
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ON THE ANALOGY BETWEEN SMALLPOX AND CANCER.

By H. R. Gaylord, M. D.

The idea that certain points of similarity exist between the cancerous process and the acute exanthemata, especially smallpox, is by no means new. Attention has been called to the subject from two entirely different points of view, both, however, having as their basis the infectious nature of cancer. Those who have been engaged in the study of the cell-inclusions of cancer and vaccine and find in these inclusions certain points of resemblance were the first to call attention to the analogy existing between the two problems. The second point of view is as yet represented by Borrel, who has recently attempted to show that without regard to the question of the inclusions (which he views as often characteristic of the process but not as parasites), certain points of similarity can be traced which lead him to the conclusion that although the nature of the agent is as yet undiscovered, both the epithelioses and epithelioma are infectious and may be said to possess a virus which is capable of transmitting the disease. He supposes the existence of a cancer virus, which is suggested to him by results recently obtained in the transplantation of epitheliomata in mice. Regarding the similarity of the virus found in smallpox and the virus obtained in sheep-pox (clavelée), Borrel brings many points of interest, especially certain experiments with the latter which is found to pass through coarser grades of the Chamberlain filter but not through the finest.

Without entering here into the details of Borrel's research, to which we shall later have to refer, we may say that he favors the idea that in both cancer and smallpox the infective agent is a minute, if not invisible parasite, and that the inclusions are the result of degenerative changes in the protoplasm or of invaginated leucocytes in process of disintegration. He finds in sheep-pox a characteristic inclusion in the cytoplasm of the epithelial cells, and in an epithelioma of the mouse which he used for transplantation, similar inclusions. The inclusions found in the corneal epithelium of the rabbit after inoculation with vaccine, he believes with Salkowski, can be reproduced by irritants such as diphtheria toxin, or superheated vaccine.

In the light of the recent work of Councilman, Brinckerhoff and Macgrath, and the elucidation and confirmation which their work on vaccine and smallpox has found at the hands of Calkins, it would be profitable to recall the points of resemblance which may be traced between cancer and these processes on the basis of the inclusions found in both.

The first author who especially called attention to the similarity of appearance between certain forms of the so-called vaccine body or Cytoryctes vaccinæ (Guarnieri) was Gorini.* This author pointed out that in inoculated corneæ certain larger forms of the vaccine body were frequently surrounded by a form of capsule, and that they then closely resembled the cell-inclusions of cancer. The forms in question in vaccine had been described previously by L. Pfeiffer, Guarnieri and Clarke and were interpreted by these authors as a more advanced stage of development of the smaller and characteristic vaccine body. A gradual transition between the typical vaccine body and these larger inclusions could be traced by Gorini, who concluded that they are developed from the smaller inclusions. These larger bodies he finds possess a high degree of similarity in form and appearance to the cell inclusions of cancer.

In 1900 we were led to conclude that the inclusions of cancer and those of vaccine were essential in their nature, although not identical, and reinforced by the publication of Wasielewski† who, to our mind

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*Gorini. Centralbl. f. Bakteriologie Abt. I, Vol. 28, 1900.

† The conclusions of Wasielewski are so important that it is not out of place here to give them in full:

Contribution to Our Knowledge of the Nature of Vaccine.—Von Wasielewski Zeitschrift für Hygiene, Vol. XXXVIII, Part 2, October, 1901.

"*Conclusions.* The vaccine bodies are the single characteristic structures which are found in the epithelial cells of the skin and mucous membrane brought all but convincing proof of the parasitic nature of the Cytoryctes vaccinæ, we arrived at the conclusion that the conditions surrounding both problems were identical and that if the inclusions in vaccine were parasites, the inclusions in cancer were likewise parasites.

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in vaccine and variola. These are absent in normal epithelial cells of the skin and under all other conditions. The bacteria described as the specific factors in vaccine are saprophytes and have no etiological significance as shown by the efficiency of lymph entirely free from bacteria.

"2. The characteristic vaccine bodies invariably appear in the corneal epithelium of rabbits inoculated with potent vaccine.

"3. The same structures can not be induced in the epithelial cells of the cornea in rabbits in any other way.

"4. It is impossible that the vaccine bodies are leucocytes or degeneration products of leucocytes.

"5. The derivation of these bodies from the nuclei of the epithelial cells is disproven by, first, their appearance in perfectly normal cells; second, their presence in cells which are undergoing mitosis; third, by the appearance of the smallest of these bodies at the periphery of the lesion, where they make their first appearance in the peripheries of the cells most widely distant from the nuclei.

"6. The origin of these bodies from the cell protoplasm as the result of the specific action of an organism supposedly invisible because of its minuteness, can not be proven or entirely disproven but is rendered throughout improbable on the following grounds: *a.* Because the specific factor of vaccine can be removed by filtration for which reason there is no ground for supposing it to be an infinitely minute organism. *b.* Because the vaccine bodies appear in cells undergoing division in which the karyokinetic figures show the characteristic protoplasmic radiation and the figures themselves are derived from the central portion of the cytoplasm, which according to the views of Hueckel is first affected by the specific action of vaccine. *c.* Because a similar specific transformation involving alone special portions of the protoplasm has never been proven in any other case. *d.* Because the extensive and rapid appearance of degeneration and vacuole formation as that of the granulated degeneration of the vaccine bodies has never been observed in any of the degeneration processes of the protoplasm, because the development of such characteristic cell-inclusions can not be compared with any known form of cellular degeneration. *e.* Because the impotent filtrate of vaccine lymph has no specific toxin action upon the epithelium.

"7. Size, form and structure, distribution and extension at the point of inoculation, as well as the appearance of evidences of division and degenera-

Our analogy extended likewise to the examination of fresh material in which we found certain hyaline bodies which we compared to the so-called fresh vaccine body.

On the same date of the same year Bosc* published an article advancing exactly the same idea but including sheep-pox, a disease which we had not been able to study. Bosc went so far as to classify these diseases and believes that they form a group of sporozoan infections. Bosc gives an exhaustive description of sheep-pox, the virus of which causes epithelial lesions and connective tissue nodules in the subcutaneous tissues. In the exudate obtained from fresh postules he found characteristic epithelial cells containing highly refractive bodies embedded in the protoplasm and similar free bodies, many very small, which presented characteristics similar to the inclusions in the cells. He finds that some of the inclusions and free bodies take the nuclear stain, although irregularly. In

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tion forms, speak for the supposition of Guarnieri that the vaccine bodies are parasites (schmarotzi) within the cells.

"8. The changes which the presence of the vaccine bodies induce in the cells support this view.

"9. The propagation of vaccine through forty-eight generations by inoculation on the cornea of rabbits proves that an active continuous proliferation of the vaccine bodies occurs at the site of inoculation.

"10. That, besides the vaccine bodies at the point of inoculation, no other organisms are either microscopically or bacteriologically demonstrable, and the constant reappearance of the vaccine bodies to the forty-eighth generation is demonstrable."

The author concludes that the supposition of Guarnieri that the vaccine body is the vaccine organism must be viewed as extremely probable.

The importance which we attached to Wasielewski's work and our point of view is shown by the following paragraph taken from the manuscript of an address given by us in Detroit February 21, 1902, before the Wayne County Medical Society: "It is manifestly impossible to apply all of these conclusions to the organisms of cancer, inasmuch as the experimental basis in cancer is infinitely more limited, but by a realization of the intrinsic nature of the two problems, it may readily be seen that the definite establishment of the vaccine body as the vaccine organism places us a great step forward in an appreciation of the true significance of these characteristic cell-inclusions in cancer."

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*Bosc. Archives de Medecine Experimentale. Vol. XIII, No.3. May, 1901.

many, however, the central portion of the body takes a chromatic stain. Similar inclusions are to be found in the cells forming the connective tissue nodules. *The inclusions vary in size from bodies not larger than a micrococcus to large structures either spherical or of irregular form with a more refractive central body.* All the inclusions are surrounded by a more or less clearly defined zone of clear protoplasm. Bosc inoculated the cornea in a number of sheep and obtained a lesion similar to that of vaccine, the inclusions being very abundant by the sixth day. As controls he inoculated the cornea with pathogenic bacteria, fungi, dust and *superheated sheep virus.* In no case were inclusions formed which could be compared to the inclusions following inoculation with the fresh virus.

Without going further into the details of Bosc's paper, to which the reader is referred, it may be seen that he definitely establishes the presence of characteristic inclusions and shows that in this connection the virus is similar to that of vaccine. Neither Wasielewski nor Borrel describes intranuclear inclusions in vaccine or smallpox, but Bosc* has since published a paper on the parasite of variola which appeared the 24th of October, 1903, describing practically all the forms observed by Councilman. His paper, although appearing several months after Councilman's preliminary announcement, affords an independent observation of these structures. Thus far in sheep-pox no intranuclear forms have been observed.

The only reference to nuclear inclusions in vaccine, which in the light of Councilman's work receives a renewed interest, is that of Gorini.† Gorini found that certain forms of the Cytoryctes vaccinæ could be found within the nuclei of the epithelial cells of the inoculated cornea, and that many of the inclusions were so placed in relation to the nucleus that they must be looked upon as either penetrating or being cast off from the nucleus. Many of the intranuclear inclusions are of the larger variety; i. e., those already referred to as closely resembling the inclusions of cancer. Gorini concluded that either the Cytoryctes is a parasite which infects the nucleus and in many cases passes then to the protoplasm (or the reverse), or that the Cytoryctes is not a parasite but is a characteristic body derived from the nucleus. Which of these hypotheses he favors Gorini does not state.

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*Bosc. Comptes rendus des Séances de la Société de Biologie T. LV. p. 1204.

†Gorini. Centralblatt f. Bakteriologie. Abt. I. Vol. 29, 1901.

The presence of typical birdseye inclusions within the nuclei in cancer was first noted by Steinhaus.* A simple reference to his illustrations clearly defines this point. Plimmer† likewise found the same inclusions in nuclei and gave an illustration representing this phase. Their presence has also been noted, described and illustrated by Apolant and Embden, who in contradistinction to the above-mentioned authors, who held them to be parasites, believed that many of the birdseye inclusions are derived from the nucleus as the result of degenerative changes. In fact, Apolant and Embden believe that the birdseye inclusions in the protoplasm and the birdseye inclusions in the nuclei are produced by different processes and can not argee with Nösske that the only source of the inclusions is the degenerated protoplasm. This criticism applies to Greenough, who preceded Nösske in attributing these inclusions to secretive processes in the protoplasm. The presence of inclusions in the nucleus likewise diminishes the value of such experiments as those made under Aschoff's direction by Spirlas, in which the introduction of foreign cells into the peritoneum of animals leads to the formation of inclusions in their leucocytes which present a similarity to birdseye inclusions. These experiments tend to show that the inclusions are the result of foreign bodies entering the cells from without. They must also be found in the nuclei of the leucocytes, if Aschoff's criticism is to hold. For the nucleus these authors assume that the birdseye inclusions are formed in various ways. They assume these bodies are formed within the nucleus and that the central body which is often acidophile is derived from the nucleolus of the cell, which is basophile, by some process which simply changes its staining affinity. The inclusions with acidophile central bodies are in appearance identical with the inclusions in the protoplasm. They suggested that in many cases extrusion of the nucleolus with transformation of its staining affinities might account for the formation of some of the inclusions in the protoplasm. The idea that the inclusions with acidophile central bodies might have penetrated into the nuclei from the protoplasm, in which case it is not necessary to assume a mysterious transformation of the staining affinities of the central body, does not of course occur to these authors, whose purpose is to show that the inclusions are derived from the constituents of the cells and have not entered from without.

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*Steinhaus. Virchow's Archiv. Bd. 126.

†Plimmer. The Practitioner. April, 1899.

Borrel, who appears for the present to lay less stress on the centrosome as the origin of the inclusions, believes on the other hand that in most cases they are metamorphosed leucocytes. That it is manifestly impossible to prove that an object in a hardened section is passing in either direction must be apparent. It would seem, however, that unless we assume a multiplicity of sources for these so characteristic inclusions we are not able to satisfy the many observers who have studied them from other than the parasitic standpoint. On the other hand, those who view them as parasites offer but one explanation, and are reinforced by the presence in other recognizedly infectious processes of the epithelium of inclusions likewise viewed as parasites, which they closely resemble.

In our original publication* we noted the presence of intranuclear inclusions but devoted our attention more definitely to the cytoplasmic forms. Since the publication of Councilman's article Calkins has demonstrated the frequent occurrence of typical intranuclear birdseye inclusions, thus confirming the observations of Steinhaus, Plimmer and Apolant and Embden. The work of Councilman, showing the presence of a complete cycle of an intranuclear organism in smallpox, must undoubtedly call attention to the importance and shed light on the probable significance of characteristic intranuclear inclusions in cancer. If the restrictions of Apolant and Embden are correct in the case of the intranuclear inclusions of cancer, they apply with equal force to the intranuclear inclusions of Councilman in smallpox, and Gorini in vaccine. The only essential difference in the two cases, as we have previously pointed out, is that vaccine and variola are accepted as certainly infectious and cancer is not.

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*Third Annual Report of this Laboratory for the year 1900.

The grounds on which most pathologists refuse to see in cancer an infectious process is that as yet all experiments calculated to show the infectious nature of cancer have as their basis the transplantability of the cancer cell. In the case of Borrel, however, the transplantation results appear to have brought conviction of the infective nature of the process. His belief that the inclusions both in cancer and smallpox are not parasites leads him to the hypothesis that the organism of cancer must be of ultra-microscopic dimensions.

For this reason it is well to consider what is the evidence of ultra-minute organisms for vaccine, variola and sheep-pox. The virus of sheep-pox has been shown by Borrel to pass through the Berkefeld and the coarser grades of the Chamberlain filter. Vaccine is supposed not to pass through any of the bacteria-proof filters. We are, however, informed by Dr. Georges Dryer that he has recently succeeded in passing vaccine through Berkefeld bougies which were proof against the bacillus of chicken cholera, recognizedly one of the more minute bacilli. This being the case, vaccine is placed on the same footing as the virus of sheep-pox. Mouth-and-claw disease, which is mentioned by Borrel as one of the filterable infective agents, has, however, nothing otherwise in common with vaccine and has been shown by Wasielewski never to produce inclusions or lesions in the cornea which can in any way be compared to vaccine and sheep-pox. *Should we conclude that in these diseases we are dealing with a micro parasite?*

Borrel in his publication brings light on this point. In his filtration experiments with sheep-pox he discovered that when he diluted the virus with tap water, after four days there developed in the filtered and otherwise sterile virus a small protozoon, to which he gave the name Micromonas Mesnili. The organism when in its largest form in the virus (it is of course impossible to affirm that the organism under other conditions does not possess a still larger phase), was three or four microns long and as many wide. That the organism in question had nothing to do with the virus was shown when distilled or sterilized water was used to dilute the virus. Borrel found that this organism followed the same law as the active principle of the virus, that is, it passed through the filters through which the virus passed and was held back by filters which were proof against the virus. That the organism passed through in some practically invisible spore form and then developed on the suitable medium of the virus was shown by its appearance in virus *only after four days* and the impossibility of detecting it in filtered water in which the larger forms did not develop. Borrel was forced to conclude that "Le passage à travers un filtre n'implique pas forcément l'idée d'un microbe invisible."* With these facts before him, however, Borrel pronounces against the inclusions as parasites and believes that the organism will prove to be a small if not an invisible organism.

It must be noted that Borrel's Micromonas shows as its largest form an organism considerably smaller than the larger inclusions of vaccine, variola and cancer. However, to assume that because the spore of an organism is sufficiently small to pass through a certain filter, its largest form would be within a certain limit of size, is not justified by biological knowledge. Calkins has described a protozoon, Lymphosporidium truttæ, the spores of which are one and one-half microns and divide into six sporozoites each less than one-half a micron in diameter. Borrel likewise points out that there is an essential difference between the smaller forms of motile, animate parasites and bacteria of relatively the same dimensions. The first are more plastic and accommodate themselves to the pores of the filter and pass through where bacteria are held back. In all probability the sporozoites of Lymphosporidium truttæ, less than one-half a micron in diameter, would pass through a bacteria-proof filter and yet the largest form of this organism is a multinuclear amœba twenty-five microns in diameter.

For this reason there is no ground for supposing that the filtration experiments are opposed to the protozoan theory of smallpox. What must we conclude then in the light of Councilman's and Calkin's observations? If these observations are correct we have an intranuclear and protoplasmic parasite of considerable dimensions, which, in one of its phases, passes through a Berkefeld bougie. The cytoplasmic and certain intranuclear forms described by Gorini have a recognized similarity of appearance to the cytoplasmic and intranuclear inclusions in cancer. The difficulty in carrying the analogy further is found in our inability thus far to separate from the epithelial cells in cancer an infective agent capable of producing cancer even in animals of the same species. All of the experiments in cancer have as their basis the epithelial cell, and it has thus far been impossible to apply with success filtration experiments similar to those of Borrel and others in smallpox. For this reason it is difficult to understand why Borrel, who believes in the infective nature of cancer, should seek its solution in an ultra-microscopic organism. It would appear that the closest analogy which we can draw between smallpox and cancer is the presence of the inclusions in the epithelial cells, and it would seem that filtration, even in the light of Borrel's experiments, in no way shakes the conclusions of Councilman and Calkins.

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* Without attempting to discuss all the filterable viruses it may be noted that the blood of yellow fever patients passes through the Berkefeld bougie but not the fine Chamberlain. Without regard to the work of the Marine Hospital Commission which has been so sharply criticised by Carrol, it must be noted that the relation of the mosquito to this disease strongly suggests a protozoon, perhaps an extremely small one.

We have maintained from the first that the solution of the vaccine-variola problem, with the establishment of the vaccine body as the vaccine organism, would bring, as a natural consequence, the establishment of the probable protozoan nature of the inclusions of cancer. The difficulty of the problem in cancer is unfortunately greater than in smallpox, owing to the limitation of experimental possibilities, but the advances which have been made in the study of the inclusions of the exanthemata have already thrown a suggestive light on the question of the inclusions in cancer. As the whole question stands, it must be conceded that the importance of questionable inclusions in processes with obscure etiology has greatly increased, and that the interpretation of the inclusions of cancer as parasites has gained rather than lost ground as the result of the researches of the last two years.

REPORT OF THE BIOLOGICAL-CHEMICAL DEPARTMENT

By G. H. A. Clowes, Ph.D.

A brief summary of the work carried out in the biological-chemical department since the completion of the new laboratory was presented in the Fourth Annual Report. In the absence of sufficient reliable analytical data it was decided to postpone publication of results, especially those bearing upon the metabolism of patients suffering from cancer, until a sufficient amount of confirmatory evidence had been accumulated. The work of the chemical department, either in progress or contemplated, was considered under three main heads: (1) Studies in metabolism regarding the equilibrium of nitrogenous bodies, chlorides, etc., in the system, and including work on the stomach contents, blood and urine; (2) Micro-chemical investigations carried out with a view to obtaining evidence regarding the cause of cancer and the nature of the disturbances taking place in the metabolism of the individual cancer-cells; (3) Attempts to cure cases suffering from cancer by means of serum-therapeutic treatment or chemical agencies, the possible value of which had been indicated by investigations carried out under the two first heads. To these three main lines of work must be added a fourth, to which a considerable amount of attention has been paid in the course of the last two years; the examination of cancerous material and the serum of cancer cases for hæmolysins, precipitines, etc., applying the principles evolved in immunity studies carried out in other directions.

The work included under the heading of metabolism is practically completed and the results obtained are to be found in a series of papers published in this report dealing with the nitrogen and chlorine equilibrium established between the food and excreta of cancer cases, and the influence of varied proportions of salts in the diet upon the blood and urine. In this portion of the work special attention has been paid to the question of improving the nutrition of patients suffering from tumors which from their nature and location are capable of interfering with the proper assimilation of food.

A paper has also been included dealing with the secretions of the stomach in cancer as compared with normal and other pathological conditions and indicating to what extent chemical analysis of the contents of that organ can be utilized for diagnostic purposes, and also endeavoring to throw light on the nature of the disturbances in osmotic equilibrium which lead to an interference with the normal secretions.

In a paper dealing with the possibility of diagnosing cancer by considering the nature and proportion of the various urinary constituents it is pointed out that any indications obtained by this means regarding the location of a tumor are purely the result of secondary interferences with the metabolic equilibrium of the cells of the organs involved, and that no specific cancer reaction can be said to have been obtained by this means.

Abstracts of papers published in other journals dealing more particularly with the physico-chemical problems involved in the osmotic equilibrium of a variety of pathological cases have been included.

Throughout the work referred to above an effort has been made to present a thorough summary of the literature to date of publication, and to point out wherein the work carried out in this Laboratory is in agreement with or deviates from that of previous investigators.

Under the second head of micro-chemical research a paper dealing with the staining reaction of mast cells, etc., has already been published, and further work of this nature dealing more especially with the cancer-cells themselves is already in course of completion. Special effort is being devoted to the problem of obtaining an immunity against cancer, and horses are being employed for the preparation of sera. The results obtained in this direction are not, however, sufficiently positive to permit of any immediate expression of opinion regarding the ultimate probability of success. The possession of a series of cancer mice, kindly put at the disposal of this Laboratory by Professor Jensen, has made it possible to commence experiments on immunity on a much larger scale and with much greater likelihood of success than was possible when dealing with human cases exclusively.

In view of the interest attaching to the employment of X-rays in the treatment of cancer, a short note on the influence which they exert on the metabolism of normal animals has been incorporated in this report.

A series of analyses of mouse tumors taken at various stages of development is being carried out in this Laboratory at the present time. Such tumors, having an absolutely common source, afford a much more satisfactory basis of comparison than can be hoped for from a series of human tumors.

Finally, with a view to possible early diagnosis, tumors and the blood sera of cancer cases have been examined for hæmolysins, precipitines, etc. Whilst certain positive results have been obtained, it has not at present been possible to prove conclusively that the reactions in question were directly dependent upon the cancer, or the result of some secondary interference. With the cancer mice at our disposal it is hoped that we shall be able to obtain positive results bearing on the question of immunity and serum diagnosis, to which it is proposed to devote the special attention of this Laboratory during the forthcoming year.

STUDIES IN CANCER METABOLISM.

By G. H. A. Clowes, Ph. D., W. S. Frisbie, Ph. B., and H. H. Glosser, M. D.

A review of the literature published in the course of the last fifty years on the equilibrium of nitrogenous bodies and salts in cases of impaired metabolism makes it apparent that the most divergent views are held on this subject by different investigators. Marked disturbances in the secretion of urea and chlorides in cachectic cancer cases were first observed by Vogel, Rommelaire, Jacoby and others, but these early investigators made very little attempt to carry out strict analyses on the food stuffs consumed in comparison with the waste products excreted. Müller and Klemperer (to whose publications a detailed reference will be made later) came to the conclusion that under the influence of toxic bodies produced from tumors a loss of nitrogen was suffered by the body, while Moraczewski in the majority of his cases finds a retention of nitrogen in the system, the amount contained in the food being in excess of that secreted in the urine and fæces. Similar differences of opinion are to be noted in the works of Müller, Laudenheimer, Schöpp, Bouveret, Böhne and Moraczewski on the retention or secretion of chlorides. A considerable increase in the proportion of sodium chloride in the blood of certain cancer cases has been observed by Moraczewski, and it has even been claimed that greatly increased quantities of chlorides are retained by certain organs of the body, especially in those cases in which an œdematous condition is to be observed. In many of the cases investigated disturbances in the equilibrium of salts were found to be associated with an impaired secreting function of the kidney, which would make a clear differentiation between cause and effect extremely difficult. Great advances have been made in physical and physiological chemistry since the publication of the work of these early investigators. The laws of osmosis governing the equilibrium between soluble salts, the body fluids and the cells render it extremely improbable that large quantities of sodium chloride, for example, could be retained in the system, as has been claimed by some writers, without the retention of a corresponding quantity of water necessary to maintain, at any rate to a certain extent, the osmotic equilibrium, which would cause an increase in body weight. The ability of certain electrolytes to aid in the removal of products of suboxidation from the system, has long been recognized and more recently the studies of Köppe, Hamburger, Gerber, etc., on the metabolism of blood cells have demonstrated the significant role played by chlorine ions in the removal of products of oxidation from the cell. It is perfectly conceivable that toxic bodies produced from a tumor might seriously interfere with normal osmosis; also that the abnormal products produced through the disintegration of cells and the influence of toxins might prevent to a certain extent the dissociation of salts, thus interfering with their normal function and at the same time reducing the osmotic tension which they exert and lead to their retention in larger quantities in the system, such a process being accumulative in its action. Loeb, Matthews, Calkins, etc., have shown how significant is the role played by electrolytes in the stimulation of individual cells, and the possible influence of disturbances in osmotic equilibrium of the cell leading to its proliferation can scarcely be ignored.

In this paper it is proposed, in the first place, to summarize the existing literature bearing on the problem of nitrogen and chlorine metabolism in cancer as compared with the normal, and subsequently to record some results having a bearing on this subject obtained in this Laboratory in the course of the last two years. Whilst our main object in carrying out these experiments has been to find some means of improving the nutrition of cases in which tumors have directly or indirectly interfered with the proper assimilation of food, and also to study the effects exerted by various salts and chemical bodies when taken in conjunction with the food or on direct injection, the exact analyses carried out from day to day on the diet, urine and fæces, and frequently on the blood, enables us to throw some light on the discrepancies observed in the literature and to arrive at definite conclusions which will be referred to at a later stage.

In reviewing the early literature one of the things that first impresses itself on one's mind is the absence of accurate data regarding the diet, the frequent disregard of the loss of nitrogen and chlorine in the fæces and the failure to record variations in the weight of patients. It should be possible to obtain a more or less accurate balance between the increase in weight of the tumor, the loss of body proteid, the formation of œdematous fluids and all such factors, when considered in conjunction, with the differences between intake and output of nitrogenous bodies and salts in a given period of time. In work of this nature the sources of experimental error, especially in the hospitals, are, as Müller points out, extremely numerous. Our own experience has taught us that unless the intelligent cooperation of a patient is obtained, or at least of all those immediately concerned with the charge of the case, it is perfectly useless to look for even moderately reliable results, however accurate the work in the laboratory may be. It is very probable that careless patients, especially those capable of moving freely about the wards, have deceived those who were interested in studying their metabolism, not only as regards their diet but also with reference to the twenty-four-hour secretion of urine, a fact which might well explain the contradictory results obtained by different authorities. One surprising source of error frequently met with in the literature is to be found in the change from a solid to a liquid diet. Milk without any addition of chlorides contains considerably less chlorine in proportion to the nitrogen than does the normal diet, consequently a diminished secretion of chlorides would be observed in the urine, and is not necessarily in any sense attributable to the influence of a tumor or other pathological cause.

Vogel, who was the first to investigate quantitatively the constitutents of the urine of a case of cancer of the liver, observed a daily secretion of urea under 8 grams and assumed that an actual retention of proteids took place in the blood in excess of those consumed in order to provide the materials required by the tumor cells in their development. Since, however, no records of the diet, of the nitrogen content of the stools or the body weight of the patient are recorded, this conclusion is scarcely justifiable. Rommelaire, in cases of cancer of the stomach, and Gregoire confirmed Vogel's observation of a greatly diminished secretion of urea, but these investigators seem also to have overlooked the probable diminution of proteids in the diet, and whilst reference is made to diarrhœa, no account seems to have been taken of the loss of nitrogen by that source. Robin expressed himself as opposed to the views held by the above-named writers, claiming that in well-nourished cancer cases the urea secretion was normal, and assumed that the diminution of nitrogen in the urea was simply to be attributed to a diminution of the quantity of proteid in the diet. Dujardin-Beaumetz supports Robin, finding in certain cancer cases high urea secretion, reaching 17 and 25 grams per diem, which he considers may be attributed to the influence of toxins secreted by the tumor, whilst certain nonmalignant tumors show results as low as 4 grams per diem.

The first work of this nature which can be said to have any real value was published by Jacoby in 1887. This author compared the daily nitrogen excretion of a normal individual with that of a case of carcinoma of the liver associated with jaundice, both cases receiving identically the same diet. He succeeded in demonstrating that whilst a normal individual could be made to gain weight and secrete less than 12 grams of nitrogen daily, the cancer case would ose weight and as much as 18 grams nitrogen would be eliminated n the urine.

The next paper on cancer metabolism possessed of any considerable value was published in 1889 by Friedrich Müller, who made an exhaustive study of the proteid equilibrium in a series of nine cancer cases, in some of which nutrition was mechanically interfered with by the tumor, and compared the results so obtained with experiments previously carried out by himself and other investigators (Senator, Monk and Zunz) on starving individuals. The conclusions arrived at by Müller are extremely interesting. He succeeded in demonstrating that in all his cases in spite of a rapid development of the tumor more or less loss of nitrogen was suffered by the body as a whole when the heavy loss of that substance in the fæces was taken into account. He also proved that this loss was more or less proportionate to the loss of body weight when due allowance was made for the formation of œdematous fluid. He further demonstrated that in those cases in which the nutrition of the patient was seriously disturbed by the developing tumor in such a manner that the loss of nitrogen by the fæces was practically equal to that contained in the food, the condition of starvation thus established was very comparable with that obtained in his experiments on starving normal individuals. In studying the influence of toxins produced by the tumor, Müller made one very valuable observation to the effect that whilst the average loss per kilo of body weight per diem was in the case of a series of starving healthy individuals only .103 grams nitrogen, that of four cachectic cancer cases amounted to .175, .225, .166 and .146 respectively. He also noted that even in one case in which the appetite of the individual under experiment was considerable, it was still found impossible actually to maintain the nitrogen equilibrium, although the amount of nitrogenous material assimilated was far in excess of that which would have been required by a normal individual of the same weight. He notes similar results in a case of typhoid during the early period of the disease and whilst the patient was suffering from toxic symptoms, the exactly reverse results being obtained during the period of convalescence. Müller concludes that in all cases of cancer the destruction of proteid suffered by the body owing to faulty nutrition and toxic effects is greater than the increase in proteid of the growing tissue of the tumor, and that consequently a loss of nitrogen is always suffered by the system. He attributes the coma of carcinoma to auto-intoxication through the products of metabolism which have not been removed owing to faulty functioning of kidneys affected by toxins. It is only to be regretted that this extremely accurate worker failed to take account of the chlorides present in the diet in order that they might be compared with those of the urine and fæces. In his analyses of urine the quantity of chloride was frequently extremely low, and he concluded without much ground that this signified the destruction of body proteid rather than circulating proteid. Sticker and Huebner observed a much higher secretion of sodium chloride in nonulcerating carcinoma than was the case in ulcerating carcinoma of the pylorus. G. Klemperer, who carried out fairly exact metabolism experiments on seven cancer cases, confirms the original observation of Jacoby, that when normal individuals and cancer cases are submitted to the same conditions of experiment, the former may gain whilst the latter lose in body proteid, a difference in nitrogen content of the food and excreta as high as 7.5 grams daily being observed. Huebner considers that the diminution in the alkalinity of the blood, and the fatty degeneration of organs, associated with increased tissue destruction, may be looked upon as evidence that carcinoma is an intoxication disease, and that the coma associated with the last stages may be attributed to the action of the poison derived from the tumor circulating in the blood and acting upon the central nervous system. Bouveret attempted to differentiate between diseases associated with hyper-secretion of hydrochloric acid in the stomach and cancer of the stomach by means of the secretions of chlorine in the urine, claiming an increased excretion associated with diminished secretion of hydrochloric in the stomach. This finding was not in agreement with that of Stroh, who observed a diminution in the chlorides secreted in both cases. Laudenheimer, experimenting on five cancer cases with reference to the chloride equilibrium, obtained in two cases a secretion of chlorides comparable with the consumption and in two other cases a marked diminution of the secretion of sodium chloride in comparison with the consumption. This author concludes that the disturbances in chloride equilibrium are the result of nephritic or circulatory retention of water and salts. Schöpp in a couple of papers claims that whilst the excretion both of chlorine and nitrogen frequently falls below the amount assimilated in cancer cases, the difference may generally be attributed to ulceration of the tumor, the secretion containing considerable quantities of sodium chloride. In a case of cancer of the stomach and one of breast carcinoma, equilibrium was maintained in the absence of ulceration.

From 1895 to 1897 a series of important papers were published by Moraczewski on the blood and urine in cancer, on metabolism in carcinoma and chlorisis, and subsequently on leucæmia and pseudo-leucæmia. In the first paper on the chlorine and phosphorus content of the blood and urine in cancer, after referring to the literature, the author estimated phosphorus, chlorine and nitrogen in the blood and urine of cases of carcinoma, anæmia and other pathological conditions. He found a reduction in the phosphorus content of the blood from the normal of .1 per cent to .07 and .04; chlorine, on the other hand, was high, amounting to as much as 4 per cent of the dried substance. He remarked further that as the chlorine in the blood increased the chlorine in the urine decreased in cases of carcinoma, but no particular reference was made to the condition of the kidneys. He considered that the nitrogen content was also increased in proportion corresponding with the increase of chlorine in cancer but not in anæmia. Moraczewski's work on cancer metabolism published in 1897, eight years later than that of Müller, gives results which are in no sense in accord with those obtained by the latter investigator. Whilst Müller observed a heavy loss of nitrogen in a long series of cases, Moraczewski found that large quantities both of nitrogen and chlorine were retained by the system, and at the best an approximate equilibrium was established. In none of his cases was a loss of nitrogen recorded over any extended period. The amount of nitrogen and sodium chloride retained in the system in a short space of time was frequently vastly in excess of the amount which would correspond with the changes in weight of the patient. In fact, in certain cases it is apparent that there must have been some source of experimental error, the quantities secreted being so far below those assimilated that either the patient must have gained ten or twenty kilo instead of, as was actually the case, undergoing very slight changes in weight, or chlorides must have been deposited in the tissues, which is practically impossible. Were it not for these discrepancies this work of Moraczewski's would be a most valuable contribution to the study of cancer metabolism. This author observed the proportions of nitrogen, chlorine, phosphorus and calcium in the twenty-four-hour urine whilst subjecting the patient to the influence of various salts, the diet being maintained on as uniform a basis as possible. He concluded that in cancer large doses of sodium chloride or calcium phosphate may be used to effect a saving of proteid whilst they stimulate the excretion of chlorides. He failed, however, to take account of the probable retention of waste nitrogen products in the œdematous fluids resulting from the presence of large quantities of these salts. He found that silver nitrate given in small doses increased the secretion of nitrogen and phosphorus and decreased that of chlorine and calcium, chlorides being retained as might be expected. Silver nitrate, however, produced diarrhœa and other acute symptoms in some of the cases to such an extent as to be discontinued. More especially was this effect to be noticed in anæmic cases. He concluded that the retention of chlorides is increased with the increase of anæmia, which is in accord with the supposition that such a retention of salts might merely represent the dilution of blood or body fluids which takes place under these conditions. He also found that phosphorus is retained in conjunction with chlorides in spite of the fact that phosphorus is present in the blood in proportions which are frequently the inverse of those of chlorine; also that the secretion of calcium is inversely proportional to that of phosphorus, and that a larger amount of calcium is retained in slightly anæmic conditions. In one case, for example, the addition of ten grams of sodium chloride daily to the food led, as would be expected, to an increase in the retention of chlorine and nitrogen, whilst the entire elimination of chlorides from the diet and the addition of ten grams of calcium phosphate led to a considerable chlorine loss. On the other hand, .1 gram of silver nitrate resulted in what was practically a balance of the proportions of nitrogen and chlorine, a much larger amount of chlorides being retained than during the treatment with calcium phosphate. Ten grams of sodium phosphate led to a retention of nitrogen and calcium, chlorine remaining about normal. Five grams of sodium chloride and 5 grams of potassium chloride led to a retention of nitrogen and chlorine and a proportionate loss of calcium and phosphorus. On comparing cancer cases with those of chlorosis he concluded that the latter are capable of taking much larger doses of potassium chloride than the former without noticeable toxic effect. Whilst an immense number of results were recorded in this paper, their failure to balance and a want of agreement in some cases detracts from their value.

Moraczewski also published a series of experiments on the chlorine, nitrogen, phosphorus and calcium equilibrium in a case of leucæmia and the data which he obtained extending over a period of six weeks, whilst the patient was subjected to a variety of different treatments, would indicate that a very considerable retention of nitrogen and chlorine in the proportions respectively of 40 and 30 per cent of the diet had taken place, a result which is most remarkable when it is borne in mind that in spite of the apparently rapid development of the leucæmic tumor no great gain in weight had been experienced by the patient.

Böhne found in a marked cachectic cancer case an average retention of 3 or 4 grams chloride daily for the space of four weeks, and claimed to have observed on post-mortem examination of the liver a sodium chloride content four times as great as that in the livers of three other patients. His observation has been supported by that of Achard and Loeper, who claim that the salt content of the muscle tissue of œdematous cases (non-cancerous) in which the chlorides are strongly retained, can be as high as 4.1 to 5.6 grams per 1000 in comparison with the normal, which is 1.6 to 2.8 grams per 1000; also that the brain substance, which normally contains 1.1 grams per 1000, may under conditions of abnormal retention give figures as high as 4.3 per 1000.

Cetti, in a publication concerned principally with the relative proportions of nitrogen constituents in the urine, found in two cases a secretion of nitrogen in excess of the consumption; in two other cases an ability to establish a fair equilibrium. He concluded that an increase in the proportion of extractive nitrogen is always associated with a decrease in the proportion of urea nitrogen as the disease progresses. He simply observed with reference to the chlorides that the total secretion was low, except in the case of one patient, who was receiving a full, normal mixed diet.

Koziczkowsky, in a valuable paper on the equilibrium of chlorides, refers to the frequently observed diminution in the excretion of chlorides in fevers (with the exception of malaria) and in carcinoma. The experiments recorded in this paper are directed principally to the solution of the problem as to whether chlorides, and to a certain extent other soluble salts, play a direct or indirect role in nephritis and œdema. He concludes that the retention or secretion of chlorides does not follow the same laws as those which govern the removal from the body of the products of metabolism, urea and other nitrogenous bodies, phosphates, sulphates, etc., but fails to bring very much evidence to bear on the question as to whether the retention of chlorides is due to their association in the system with katabolic products, or to disturbances in the osmotic equilibrium and mechanical forces of the body, and the ready diffusion through cell membranes, factors which might prevent the normal removal of salt. Engelman, in a communication on the osmotic pressure of body fluids, records results considerably above the normal in certain cachectic cases, but such results are generally attributable to heart and kidney insufficiency.

Braunstein, in a publication* dealing with the secretion of chlorides, phosphates, nitrogen and ammonia in carcinoma, after reviewing the literature, gives the results of a series of analyses carried out on the urine of 10 cancer cases, in five of which an exact record has been kept of the nitrogen and chlorine of the diet. In three of the five cases a loss of nitrogen was to be observed. In one case of carcinoma of the œsophagus the influence of the tumor on the destruction of proteid was very marked, the average daily loss for a period of four days being 10.6 grams nitrogen. In this case the chlorides were extremely low, both in the diet and in the excreta, no great difference being observed. Numerous experiments carried out on healthy individuals in a state of inanition show a loss of only three to five grams nitrogen daily, consequently any such loss as that recorded in the case referred to must be due to toxic action leading to an abnormal destruction of proteid. Braunstein failed to find any considerable differences between the salt content of the diet and the excreta, in certain cases the latter even exceeding the former. His experiments with diuretin led him to conclude that the sodium chloride secretion was directly proportional to the urine secretion, and that the accumulation of chlorides observed in certain cases was simply attributable to the failure of the kidneys to remove water, and with it the salts held in solution. He further states that in cancer an increase rather than a decrease of the secretion of chlorine in the urine may be looked for provided the kidney function is satisfactorily performed.

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*This publication was received in June, 1904, after the completion and shortly before the publication of this paper.

To summarize the literature on this subject, it may be said that widely differing results have been obtained by different authors, as regards the nitrogen and chlorine metabolic equilibrium of cancer cases. Whilst on the one hand it is claimed that nitrogen is retained in the system as a result of the tumor development and the demand created thereby for proteids, on the other hand it is shown that in spite of the tumor growth the nitrogen loss suffered by the system due to the destructive influence of toxins on the body proteids is very considerable except in those cases in which a marked œdema has developed, and in which the failure of the kidneys to function has led to an abnormal retention of waste nitrogen. Whilst numerous authors claim to have observed a diminution in the total amount of nitrogen and chlorine of the urine, especially in the last stages of cancer, few of the early writers regarded sufficiently diminutions in the proportion of chlorides in the diet, especially in those cases in which patients were no longer capable of assimilating solid food. Analyses of organ and body fluids showing an extraordinarily high content of chlorides, and records of the retention of enormous quantities of salt in the system far in excess of the amounts which would correspond with the observed variations of body weight and the laws of osmosis, lead us to suspect some source of experimental error, especially in view of the great difficulty in such work of properly controlling the individual submitted to experiment.

The possible importance of the chlorides in the establishment of metabolic equilibrium is more readily appreciated at the present day than was possible at the time at which most of the experiments referred to above were carried out. The intervention of modern physico-chemical theories of solution in physiological problems has demonstrated the important role played by individual ions and the dependence of any such influence upon the concentration and degree of dissociation of the compound from which they are derived. It has long been known that in fevers and other morbid conditions the equilibrium of the chlorides in the system is materially interfered with. This action may be due in part to mechanical causes brought about indirectly by the action of toxins on the cells of the heart, kidneys and other organs more especially concerned with the balancing of pressure, and consequently to a certain extent of diffusion and osmosis in the system. It may also be attributable to the action of toxins and of toxic products of faulty metabolism capable of clogging, so to speak, the normal functions of the salts in the system by preventing their proper dissociation into ions. The effect of toxins and foreign bodies upon the protoplasm of those cells of the stomach and kidneys, for example, which are capable of exerting a so-called vital secretory action on chlorides, etc. (not necessarily, so far as our present knowledge is concerned, in direct accord with the laws of osmosis), resulting in an interference with its normal function, its degree of viscosity and permeability, is a problem still open to investigation. These and many other possibilities must eventually be carefully considered, but bearing in mind the innumerable observations in cancer cases of interference with the secretions of the stomach, of increase in the chlorides and diminution in the alkalinity of the blood, of increase in the osmotic pressure of blood (Engelmann) and tissues (Hemmeter), of changes in proportion of salts in the tissues, of the production of œdematous fluids and of the diminution in the secretion of chlorides and variation from the normal rise and fall of chlorine secretion in the course of the day (Alb. Müller), it seems a matter of the first importance to throw as much light as possible upon the nature of the interference exerted in such cases, especially in view of the recent work on blood showing the role played by chlorine ions in the processes of oxidation and removal of waste products from the cells. The further possibility of variation in the proportion of potassium, sodium and calcium and other ions having a direct effect upon the proliferative tendencies of certain cells (referred to above) gives an added importance to the frequently ignored problem of salt equilibrium.

The main objects of our investigation have been as follows:

(1) To determine whether the change in weight of cancer patients was associated with a corresponding loss or gain of nitrogen by the system, and if so to what extent such results compared with those obtained from the study of normal individuals.

(2) To compare the chlorine of the food with that of the excreta with a view to discovering whether any absorption of chlorine by the body takes place, and if so to what extent.

(3) To determine whether the balance of chlorine retained in the system obtained by deducting the amount present in the excreta from that present in the diet possesses any direct relationship to the supposed increase in the chlorides of the blood and the formation of œdematous deposits; or whether the assumption of a diminished chloride dissociation can be made to account for the phenomena observed.

(4) To study the effect of adding or totally excluding chlorides from the diet as compared with the effect of such a treatment upon a normal individual.

The analytical methods employed were nitrogen by the Kjeldahl process, sodium chloride by the Salkowski or Moraczewski method, ammonia by the procedure of Schlösing, uric acid by the Hopkins method, phosphates by titrating with uranium nitrate solution and other constituents of the urine as described in the text.

METABOLISM EXPERIMENTS.

CASE I. NORMAL INDIVIDUAL.

The absence of accurate data in the literature regarding the influence of varying proportions of chlorides and other salts upon the metabolism of normal individuals rendered it advisable to carry out a series of experiments on one of ourselves which might afford a basis for comparison with some experiments made on individuals suffering from cancer. Dr. G.; physician; age 32; weight 66 kilo; health normal, received for two separate periods (one of 13 days, the other of 9 days) a diet in which the exact amounts of nitrogen and chlorine were carefully determined. In addition variations in body weight were recorded and the total volume, specific gravity, nitrogen, chlorine, phosphorus, uric acid and ammonia of the urine and the nitrogen and chlorine of the fæces were estimated. For the first five days a comparison of the nitrogen content of the food and excreta showed that a state of equilibrium might most readily be maintained on an average consumption of proteid containing from 13 to 14 grams nitrogen per diem. For the remainder of the first period and the second period considerable variations in nitrogen and chlorine content of food were accompanied by corresponding variations in the proportions of those substances in the excreta. The tendency to establish a state of osmotic equilibrium may most readily be recognized by drawing curves representing the variations in body weight, nitrogen and chlorine of the food and nitrogen, chlorine, uric acid and ammonia of the urine recorded in Table I. The weight of the body was found to be far less affected by the variations in nitrogen than by those in chlorides. Any large increase in the amount of proteid food consumed in excess of 14 grams nitrogen was apparently only partially removed by the urine, and whilst an increased amount was eliminated by means of the fæces, a considerable portion was apparently retained in the system. For the last two days of the first period a considerable reduction of the proteid of the diet (showing respectively 9.1 grams and 5.1 grams nitrogen in the food), led to interesting results. Whilst the reduction of the food from about 13½ grams to 9 grams nitrogen led to a fall of the total nitrogen of urine and fæces from about 13½ to 11.3, a reduction of the total nitrogen of the food to 5.1 in the last day was accompanied by a total nitrogen excretion of 9.3 grams. An examination of these figures shows that the reduction in the nitrogen of the food is accompanied by a proportionate loss in nitrogen on the part of

TABLE I.

First Period.

DATE.	Nitrogen in food.	Nitrogen in urine and fæces.	Sodium chloride in food.	Sodium chloride in urine and fæces.	Body weight in kilgms.	Uric acid.	Ammonia.
January.							
24–25	14.0	14.1	......	......	65.9	.58	......
25–26	15.3	15.0	......	......	65.1	......	......
27–28	12.4	13.5	7.9	8.1	65.4	.33	.87
28–29	15.5	13.7	8.1	7.9	66.1	.34	.80
29–30	13.9	15.9	27.6	20.0	66.4	.74	.63
30–31	13.9	14.2	23.0	23.6	66.5	.51	.81
31–1	17.6	14.6	12.1	17.6	66.1	.46	.76
1–2	13.0	13.1	2.0	9.5	65.1	.33	.78
2–3	9.1	11.3	1.3	3.8	64.3	.36	.60
3–4	5.1	9.2	9.3	5.4	64.7	.46	.56

Second Period.

DATE.	Nitrogen in food.	Nitrogen in urine and fæces.	Sodium chloride in food.	Sodium chloride in urine and fæces.	Body weight in kilgms.	Uric acid.	Ammonia.
March.							
30–31	12.2	10.7	......	......	66.7	.48	......
31–1	15.4	17.6	32.2	26.5	66.7	.67	.91
1–2	12.8	13.2	30.3	27.8	67.5	.52	.83
2–3	14.0	14.1	7.8	12.8	67.0	.43	.97
3–4	11.4	13.9	6.5	9.7	66.7	.46	.76
4–5	11.6	13.9	4.1	6.8	66.4	.47	.84
5–6	12.1	12.8	6.6	6.3	65.8	.38	.69
6–7	8.2	11.3	13.7	10.6	65.7	.43	.82
7–8	6.6	11.3	3.8	7.6	66.0	.35	.57

the body, but that the loss in question is not equal to the difference between the nitrogen of the food for the day in question and the normal, but about one-half of that quantity. During the greater portion of the second period the nitrogen of the food was maintained on a fairly low level. For the first four days an average consumption of about 13.6 grams nitrogen was accompanied by a corresponding loss of 13.8. The following three days on which the nitrogen of the food was still further diminished to an average of 11.7 showed an appreciable loss of body proteid, the average of the urine+fæces being 13.5. On the last two days an average of 7.4 nitrogen in the food was associated with an excretion of 11.3 grams nitrogen, or a difference of nearly 4 grams derived from body tissue. In this case, as in the first period, it will be observed that the loss of body proteid is not equal to the difference between the nitrogen of the food consumed in the period in question and the normal average amount for a corresponding period, but represents a certain proportion of that amount, in this case rather more than half.

Owing to the difficulty of carrying such experiments further it was not possible to test the effect of still further diminishing the proteids of the food or entirely removing them from the diet for the space of another day. The figures obtained by producing curves would indicate that the amount of nitrogen lost daily in a state of absolute starvation would be from 6 to 7 grams (or .1 gram per kilo) thus exceeding the figures 3 to 5 grams given by Klemperer and Blumenthal. They would on the other hand be lower than those obtained on Cetti by Senator, Zunz, etc., which are considered to have been abnormally high, owing to the toxic influence of the tuberculosis from which it was discovered that Cetti was suffering.

These normal experiments and a second series on a case of cardio-spasmus, which will be referred to later, justify the assumption that the daily loss of an individual weighing 66 kilos would be from 6 to 7 grams in a state of absolute starvation provided no toxic influences were concerned; consequently the loss of nitrogen per kilo of body weight in excess of .1 gram per diem may be attributed to the influence of toxic agencies, a point which will be referred to at a later stage in considering the metabolism of cachectic individuals.

Under normal conditions the sodium chloride of the food and of the urine lay apparently between 7.5 and 8 grams per diem. On raising the total sodium chloride of the diet in the course of one day from 8 grams to 27.6 only 19.3 was excreted from the kidneys which, with .7 in the fæces, gives a total of 20.0, thus showing a gain of 7.6 grams by the body. The following day the consumption of 22.99 grams sodium chloride was accompanied by a loss of 22.8 in the urine and .7 in the fæces, giving a total of 23.6 for the excreta. A practical balance was thus established on this day. On the following day, when 12 grams only were consumed, a total of 17.6 was eliminated, the body thus tending to recover its normal chlorine equilibrium, a result which was completely effected on the following day when, starving the patient of chlorides, only two grams being introduced into the diet, as much as 9.5 grams was still excreted. A still further reduction in the chlorides of the food to 1.3 on the following day was accompanied by a loss of 3.5 in the urine, or a total of 3.78 in urine and fæces, thus showing a still further loss on the part of the body of salt. The following day ended the first series of experiments with an increase in the consumption of salt to the normal figure, about 9.2 grams, and a loss of only 5.3, showing a tendency on the part of the system to retain chlorides in order to replace those which had been secreted during the previous period of chlorine starvation. Very similar results are to be observed in the second period, 32.2 and 30.3 grams in the diet the first two days being associated with a loss of 26.5 and 27.8, showing a retention of 5.7 and 2.5 grams, or a total of 8.2. On the two following days a reduction of the chlorides of the diet to quantities slightly below the normal was accompanied by a loss of 5 grams and 3.2 grams respectively, or a total of 8.2 grams, thus showing an exact balance of chlorides for the first four days. On the fifth day a reduction of the sodium chloride of the diet to 4.1 grams was associated with a loss of 6.8 grams, thus showing a loss of salts on the part of the body. The same interesting tendency to maintain a balance is to be noted to the extreme end of the series, the sum totals of intake and output of chlorides being for the entire period almost identical. The chlorides retained in the fæces normally amounted to .2 grams daily, but on those days on which large quantities were consumed an increase to .71 and .77 respectively was noted. In view of a recent publication to the effect that the chlorides only pass by way of the fæces after 24 hours, it is interesting to note that the almost full effect of the large doses of chlorides were observed in the space of the first 24 hours after their administration, and that without any accompaniment of diarrhœa. The effect of large consumption of NaCl on the nitrogen excretion is marked, the maximum loss of nitrogen in each period being associated with the maximum consumption and excretion of chlorides.

The body weight was found to vary directly with the amount of chlorides consumed, or more strictly, those retained in the system. In the first period the maximum of weight, 66.4 to 66.5 kilos, was observed in the course of the two days on which the largest amounts of sodium chloride were consumed and 7½ grams retained in the body. The gradual diminution of the amount of chlorides in the diet, with a gradual increase of the proportion of chlorides excreted until the latter were considerably in excess of the former, led to an exactly corresponding decrease in the weight of the individual. The minimum weight observed in the series of experiments, 64.3 kilos, was reached on the day of maximum chloride starvation, the day on which 1.3 grams only of sodium chloride was consumed in the food and 3.8 excreted, following the day on which 2 grams in the food had been accompanied by a loss of 9.5 grams. The total loss of weight between the maximum and minimum points, slightly in excess of 2 kilos, was accompanied by a loss of chlorides amounting to more than 15 grams, which would fully account for that difference in weight, assuming that a .75 to .8 per cent sodium chloride solution were isotonic with the serum. The following day a considerable increase in the salt of the diet was accompanied, as would be expected, by a retention of that substance in the system previously impoverished and consequently an increase in weight of the body was observed, although at the moment a considerable diminution in proteid in the diet accompanied by a loss of nitrogen would normally have led to a loss of weight.

In the second period the balance is even more exact. At first the retention of 5 or 6 grams sodium chloride is accompanied by an increase in weight of .8 of a kilo to a maximum of 67.5. Subsequently a fall in weight to 65.8 was accompanied by a loss of sodium chloride of nearly 11 grams. In this period also the actual loss of nitrogen on the part of the body produced an insignificant effect upon the body weight as compared with the variations in chlorides. A comparison of the volumes of water ingested and excreted shows on the average a preponderance of the former over the latter by 500 or 600 c.c. On those days on which large quantities of salt were consumed the volume of fluid ingested was about two liters and that excreted about 1300 c.c., the big secretion of sodium chloride being accompanied by a corresponding increase in total volume of urine, the specific gravity never rising above 1.023 to 1.022. On only one day in the series was the total volume of the urine in excess of the volume of water ingested, the day on which chlorides being reduced to a minimum in the diet, a large excess of salt in the system was removed in the urine.

It will thus be seen that the data bearing on body weight and intake and output of water correspond exactly with the variations in the proportions of chlorides of the diet and excreta. An investigation of the chloride figures makes it appear probable that a period of 48 hours frequently elapses before chloride equilibrium is completely established in the system, but such an equilibrium is effected very readily in contrast to the pathological cases referred to subsequently.

Effect of variations in the proportion of chlorides upon other constituents of the urine.—On those days on which the normal amount, about 8 grams of sodium chloride, was consumed in the food and excreted in the urine, the nitrogen of the urine was about 12.5, the phosphates, estimated as P_2O_5 about 2.5, and the uric acid .33 to .34 grams, the ammonia about .6 grams. A great increase in the amount of salt in the diet led to a great increase in the total volume of the urine, a slight increase in the total nitrogen excreted, an increase in the phosphates and a very large increase in the amount of uric acid, with a slight decrease in the ammonia. The uric acid results are most instructive. Two days of a normal salt diet with a uric acid secretion of .32 and .34 grams were followed by three days on which an abnormal amount of salt was consumed and gradually excreted by the kidneys, on which days respectively .74, .51 and .46 grams of uric acid, giving an average of .57 grams per diem, were removed. It will thus be seen that during the period of large salt consumption and increased excretion of water and chlorides either an increased destruction of the nucleus of cells was brought about, leading to an increase in the excretion of uric acid, or a more complete removal of the latter substance from the circulation was effected, owing to a great increase in the amount of water and salts. This latter theory is in accord with the results published by one of us on the freezing point of the urine, which indicate a probable relationship between the excretion of small ions and of large complexes such as uric acid. Following the uric acid table further, it is interesting to note that in the next two days of chlorine starvation the quantity of sodium chloride excreted adjusts itself to the normal and is accompanied by normal amounts of uric acid .33 and .36 respectively. On the last day where the factor of starvation has to be considered an increase in the amount of chlorides and partial starvation led to an increase in the amount of uric acid, .46 grams being removed. Similar results were recorded in the second period.

The excretion of ammonia appears to be much less affected than is that of uric acid by the variations in proportions of sodium chloride, but the first day on which large quantities of salt were excreted shows a diminution in the amount of ammonia, and it may be said that, generally speaking, a slight fall in the ammonia in proportion to the total nitrogen appeared to take place when large doses of salt were employed.

Throughout these series of experiments and in spite of large variations of chlorides the relationship of the freezing point to the specific gravity was found to be far more constant than would be anticipated.*

Analyses of the nitrogen and sodium chloride content of the blood were made on April 1st at the point of maximum salt assimilation and on April 5th at a stage of salt starvation:

	Nitrogen.	Sodium Chloride.
April 1st	3.01	.402
April 5th	3.43	.427

From which it will be seen that whilst the nitrogen is greater on April 5th, the proportion of sodium chloride to nitrogen is higher on April 1st.

The effect of chlorides tending to produce œdema when administered in large doses.—The increase in body weight to a maximum under the influence of large quantities of sodium chloride and its fall below the normal on reducing the salt in the diet to a minimum whilst the proteid consumption was maintained at a fairly constant level and the nitrogen equilibrium of the system underwent practically no change, has been referred to in the discussion of the body weight and chloride balance. It was further shown that the loss of chlorides suffered by the body between the maximum and minimum weights corresponded fairly well with the difference in weight, on the assumption that a salt solution almost isotonic with the blood serum was being removed from the body. It was also noted that the variations produced by changes in the chlorides were so much more marked than those brought about by reduction or increase of the nitrogen as to entirely mask the effects of the latter on the body weight.

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*See "The relationship between the freezing point depression and specific gravity of urine, under varying conditions of metabolism, and its clinical value in the estimation of sugar and albumin," by G. H. A. Clowes, American Journal of Physiology, Vol. IX, July 1, 1903.

It is interesting to note the space of time required by the body of a healthy individual to bring about a chloride equilibrium, the figures recorded showing that this may be accomplished in from 24 to 48 hours, a point which will be referred to subsequently in carrying out some experiments on œdematous pathological cases.

*Case II. Carcinoma of the Pylorus.

J. W.; age 75; weight 51 kilo.; widower.—Gives no definite history of duration of disease. Has for months past experienced considerable difficulty in taking food. Is very much emaciated and very cachectic and is not sufficiently intelligent to give a reliable account of himself. Examination shows prominent tumor on inspection in the epigastrium, which on palpation is hard, immovable and seems to involve not only the pylorus but the tissues about it. Patient is very feeble.

Death occurred on April 2, 1903, and the autopsy revealed the following conditions: On opening the abdomen about 300 c.c. of turbid fluid was found in the region of the stomach and spleen. No general peritonitis was observed. A large tumor mass, irregular in shape, involving most of the pancreas, the entire pyloric end of the stomach and one-third of ts greater curvature, was found. The size of the mass was about that of wo fists. Neither duodenum, liver nor any other abdominal organ was nvolved. On section the pylorus showed a very small opening into the duodenum and a surface as large as a man's hand of tumor tissue ulcerating and necrotic. Pathological examination confirms diagnosis of carinoma of pylorus with metastases involving the pancreas.

For a period of twelve weeks analyses were carried out on he complete 24-hour specimens of urine and fæces. For the ight weeks immediately preceding death the total nitrogen and hlorine in the food were estimated from day to day. During his latter period the patient was for the most part on a liquid iet, which considerably facilitated compilation of data. The rincipal objects to be gained by this series of experiments were) a comparison of the nitrogen and chlorine of the food and xcreta over an extensive period immediately previous to the cease of the patient; (2) the influence of varying proportions sodium chloride in the diet; (3) a comparison of the relative

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*We are indebted to Dr. Matzinger of the Laboratory staff for the hisies and autopsies on this and subsequent cases, III, IV, V and VI.

proportions of various nitrogenous constituents, phosporus, chlorine, etc., in the urine, under the varying conditions that obtain at various stages in the development of the disease.

1. *Nitrogen and Chlorine Equilibrium.*

The results obtained have been tabulated in weekly periods showing the intake and output of nitrogen and chlorides as Na Cl and the gain or loss of these substances experienced by the body for each period (see Table II). An examination of the table shows that for the first two weeks the body was practically in a state of nitrogen and chlorine equilibrium, a total of 91.4 grams of nitrogen being assimilated and the same amount excreted, whilst 26.0 grams of chloride in the food corresponded fairly well with 25.0 in the urine and fæces without any appreciable loss being experienced in body weight. During the succeeding week, from the 18th to the 26th, 14 grams of nitrogen were lost and 2.8 grams of chlorine retained by the body, the condition of the patient showing a marked falling off. In the three succeeding weeks the body suffered a steady loss of nitrogen and exhibited an ever-increasing tendency to retain chlorine, till finally, in the 14-day period immediately preceding death, whilst the nitrogen loss was 33.7 grams, the retention of sodium chloride amounted to as large a quantity as 22.8 grams, only 3.5 grams being eliminated in the urine out of a total of 26.3 contained in the food. That is to say, over 80 per cent of the chlorine assimilated with the food remained in the

Table II.

	Nitrogen in food.	Nitrogen in urine and fæces.	Loss or gain, nitrogen.	Chlorine in food.	Chlorine in urine and fæces.	Loss or gain, chlorine.
Feb. 4–11........	45.0	49.0	— 4.0	11.8	11.7	+ .1
11–18........	46.4	42.4	+ 4.0	14.2	13.3	+ .9
18–26........	36.5	50.6	—14.1	13.0	10.2	+ 2.8
26–Mar. 5....	32.9	40.2	— 7.3	8.8	4.7	+ 4.1
Mar. 5–12........	45.9	50.4	— 4.5	12.2	4.0	+ 8.2
12–19........	38.1	56.0	—17.9	13.2	6.0	+ 7.2
19–26........	30.0	40.9	—10.9	17.8	2.0	+15.8
26–Ap. 2.....	21.6	44.4	—22.8	8.5	1.5	+ 7.0

system. In the course of the week period, from March 19th to 26th, for the space of 48 hours the amount of sodium choride in the food was considerably increased in hopes of producing an increased elimination of chlorine, and with it the products of metabolism and suboxidation to which toxic effects at that time observed in the patient might be attributed, but the only result obtained by this treatment at so late a stage was an increase in the œdema of the patient. The total of the eight weeks shows a net loss of 77.5 grams of nitrogen and a gain of 46.1 grams of sodium chloride. There are, of course, in such work innumerable sources of experimental error due principally to negligence on the part of those whose duty it is to keep an exact record of the amount actually consumed by the patient in the course of 24 hours, but having due regard for the influence exerted by all such possible causes of error there can be no doubt that whilst a heavy loss of nitrogen was suffered by the system during the last six weeks, a correspondingly large retention of chlorine must have taken place. The loss of 77.5 grams nitrogen would correspond with a loss in weight of 2¼ kilo on the part of the patient whilst the retention of 46.1 grams of sodium chloride, if present in the form of œdematous fluid throughout the body, should involve a retention of a very large amount of water, at least four to six liters, and to about 40 grams of nitrogen which would otherwise have been eliminated. A certain proportion of the chlorides in question undoubtedly were present in such fluids, for especially during the last 25 days a very marked œdema of the extremities was to be observed, and at the autopsy large quantities of fluid were found in the peritoneal and pleural cavities as well as a very considerably enlarged liver and spleen, but it is scarcely possible that the retention of such a large amount of chlorine salts can be explained on the above basis, and it is highly probable that the presence of suboxidation nitrogenous products capable of forming double compounds with sodium chloride and thereby materially interfering with its dissociation in solution may account for a considerable portion of the discrepancy. It is also probable from analyses made on the tumor and other tissues that an increase of chlorides had taken place not only in the blood and body fluids but also in the tissue cells, more especially those involved in the tumor. Analyses of blood serum taken from the heart within six hours of the death of the patient and of fluids of the brain, the pleural and peritoneal cavities showed on analyses a chlorine content varying from .38 per cent to .4 per cent; the freezing points from .61 to .64° C., both of which quantities are considerably in excess of the normal.

2. *The Influence of Variations in the Quantities of Chlorides in the Diet.*

An inspection of the figures Table II will show that the patient was practically in a state of chlorine starvation, the daily average amount being little in excess of a gram of chlorine, or 1⅔ grams sodium chloride. The experiment in the course of the week, March 19-26, during which the amount of chlorides in the diet was increased to the extent of several grams, has already been referred to. During this period the chlorides to the extent of 15.8 grams were retained in the system, and in spite of a loss of nitrogen an increase in body weight of a kilo and a quarter, accompanied by a great increase in œdema, was to be observed. It is interesting to note that during this period the nitrogen loss was less than in either the preceding or following weeks, a fact which may presumably be attributed to the retention of nitrogenous waste products in the œdematous fluids. On once more reducing the chlorides to a figure lower than in any previous week the increase in œdema was no longer as marked, but the death of the patient at the end of the week in question brought the series of experiments to a close.

3. *The Relative Proportions of Nitrogenous Constituents, etc., in the Urine.*

Since this question will be dealt with in detail in a subsequent portion of the report dealing with the diagnosis of cancer by means of the urinalyses it will not be necessary to give any

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[Note.—The secretion of the skin should not be entirely neglected, as the proportion of chlorides eliminated in that manner is much larger than is that of other constituents in comparison with the urine. See p. 100].

figures at this stage. A table has been prepared showing the relationship of uric acid, ammonia, chlorine and phosphorus to the total nitrogen in weekly periods. A steady gradual rise in the proportion of uric acid nitrogen to total nitrogen, a considerable fall in proportion of chlorine to nitrogen with a fairly steady ammonia and phosphorus proportion is to be noted.

CASE III.

Mr. U.; age 60; weight 75 kilo.; German; married; occupation brewer.—No family history of cancer. Personal history good. Consumed much beer but was not intemperate. About one year ago patient began to complain of vague pain, high up in the epigastric region, which continued with some remissions and became more and more troublesome. Very soon distinct symptoms of stenosis of the œsophagus developed, such as pain in the chest on swallowing, increasing difficulty in getting solid food through and later regurgitation of food immediately after swallowing. The pain also increased and became more continuous, and six months later definite diagnosis of carinomatous stenosis of the œsophagus a few inches above the stomach was made by his physician. Difficulty in swallowing even soft foods became more marked until finally only liquids could be passed through with difficulty. Frequent attempts at dilatation of the stricture were unsuccessful. Location of the cancerous involvement was made out definitely by œsophageal sounds. August, 1903, the occlusion was complete, so that not even liquid foods passed into the stomach and starvation was imminent. Patient suffered more from the extreme thirst than from hunger, and insisted on an operation which would give him at least relief from these symptoms. A gastrostomy was then performed and the operator was unable to discover any disease of the stomach. At the time of this examination patient has for over two months been fed through the fistulous opening. On inspection he shows no emaciation or marked cachexia. He has great difficulty in breathing and coughs violently at every attempt at speaking. This feature of his illness he complains of most. He is quite helpless and feeble, complains of headache and confusion due in part to constipation and in part to the regular use of morphia which is prescribed for the relief of pain. Physical examination shows a fairly well nourished body. Ascultation of the chest reveals no great abnormalities of the heart, but it is somewhat weak and rapid and irregular beat. In the lungs the larger bronchi are full of secretions on both sides. Rales, wheezing and shallow breathing in both; signs of pleurisy, or solidification of lung substance. Palpation of the abdomen reveals an enlarged liver pushing far over the stomach with no distinguishable signs of tumor but giving much pain on pressure, especially in the epigastric region. There is a considerable cushion of abdominal fat which makes accurate diagnosis difficult.

Course of the disease.—Patient was under observation five weeks. As stated above, he was, when first seen, dull, emotional and markedly toxic due at least in part to overfeeding of eggs and meat and a constipated condition with much flatus favored by the regular use of morphia to produce sleep and to relieve obstinate cough. A brisk cathartic had been given by his physician, and at our suggestion the amount of meat and number of eggs fed during the twenty-four hours was reduced, while more vegetable food was added so that the total nitrogen content of the meals averaged 9.5. A small dose of Carlsbad salt was prescribed to be taken every morning for a week, and his stomach was washed out four hours after each meal. When test meals were given the stomach was again washed out immediately before feeding and the food allowed to remain an hour and a half to two hours. This change was followed by marked improvement so that less sedative was required; sleep became more normal and the bowels more regular; there was less flatus and less cough. A marked improvement was also noticeable in the general appearance and conduct of the patient. He increased in weight, his color was better, he was clearer in his head, was less emotional and irritable and much stronger. At the end of two weeks he sat up most of the day, was bright and active mentally, read part of the time and was able to walk about and go out of doors on good days. This condition continued until the last week of patient's life, when increasing difficulty in breathing again became prominent, with much cough and need for sedatives and expectorants. The bowels became troublesome, toxic symptoms supervened, the heart became weaker and pulse more rapid. At the same time cachexia became marked with rapid emaciation and increasing weakness. He soon fell into a semicomatose condition and died on November 17, 1903.

Death occurred November 17, 1903.—Autopsy the following morning revealed the following conditions: The skin about the fistula was puffy and full of granulations, but with no appearance of cancerous involvement. On opening the chest the lung tissue was found normal, but there were slight pleuritic adhesions at the base of both lungs. Nothing unusual about the heart. Bronchial glands slightly enlarged and pulpy. Three inches of the œsophagus, immediately above the diaphragm, consisted of a large tumor mass completely occluding the lumen of the tube. The mass was about an inch and three-quarters in thickness and when opened was full of necrotic tissue. The cardiac end of the stomach was normal. The pylorus and the entire upper portion of the stomach showed nodules of various sizes beneath the mucosa, but no ulceration. The pancreas was normal. A few of the mesenteric glands were slightly enlarged. The liver was large, extending below the ribs and a considerable distance over the pyloric end of the stomach and contained numerous large foci of cancer varying from the size of a pigeon's egg to that of a pea. Other abdominal organs were normal.

For a period of six days exact analyses were carried out on the food and excreta with a view to determining the point of nitrogen and chlorine equilibrium, also the influence of an excess of chlorides administered in conjunction with the diet. The patient received three meals a day in the form of a semiliquid vegetable soup to which a small proportion of meat had been added. The principal object of this series of experiments was to observe the effect produced upon the general condition of the patient, the excretion of urine, more especially of nitrogen, ammonia, uric acid and phosphates, employing the diet referred to above as being most favorable, but for one period reducing the consumption of salt (NaCl) to a minimum, and in a second period increasing the amount of salt taken with the food to a quantity far in excess of the normal.

The accompanying Table III gives a comparison of the nitrogen and chlorine of the diet with the nitrogen, chlorine and uric acid of the secretions.

TABLE III.

DATE.	Nitrogen of food.	Nitrogen of urine and fæces.	Chlorine of food as *NaCl.*	Chlorine of urine and fæces as *NaCl.*	Uric acid.
Oct. 20–21............	9.58	8.11	.98	3.88	.46
21–22............	9.02	9.20	1.73	3.25	.45
22–23............	9.04	7.63	1.88	1.49	.35
24–25............	9.74	9.28	8.20	3.43	.38
25–26............	9.73	8.50	9.89	7.96	.70
26–27............	9.43	8.10	9.95	6.70	.52

The total volume of urine excreted in the first three-day period of small chlorine consumption was found to be extremely large, being about six liters. In the second period, when large quantities of salt were present in the diet, the total volume of urine was less than four liters, pointing towards a probable retention of fluid in the tissue, which was indicated to a certain extent by a slight increase in the œdematous condition of the patient.

The total nitrogen secreted in the urine showed practically no difference for the two periods. In the first 22.8 grams were eliminated and in the second 22.75; thus showing that the reduced volume of urine had not materially affected the removal of nitrogenous products. To the nitrogen secreted with the urine must be added that which was found in the fæces, amounting in the first period to 2.1 and in the second to 3.1 grams, giving totals of 24.85 and 25.9 respectively, which, when deducted from the nitrogen of the diet, 27.75 and 28.9 respectively, give differences of 2.9 and 3 grams, or an average retention by the system of one gram of nitrogen per day, which must be attributed to the improved state of the patient and possibly to a certain extent to the increase in œdema; but it is interesting to note that the amounts retained are exactly the same for the two periods in spite of the much larger retention of water and salt in the latter period.

Whilst in the first three-day period the total sodium chloride consumed with the food was only 4.6 grams, the sodium chloride secreted in the urine was equal to 8.24 grams and that of the fæces to .4 grams; in the succeeding three-day period, when the chlorine of the food equaled 28 grams, the amount secreted in the urine was only 17.64 grams and that in the fæces .45 grams, showing a marked tendency on the part of the system to maintain a chlorine equilibrium more or less following the laws of osmosis between the chlorides of the system dissolved in the blood, tissues, etc., and those of the kidney secretions. An examination of the amounts lost from day to day emphasizes this point, a fall to a minimum being observed at the end of the starvation period and a rise to a maximum in the course of the following three days of chlorine excess. But it should be observed that the total amount eliminated from the system in this period of time was only 26.7 grams, whilst 32.6 were introduced with the food. This tendency to retain a portion of the chlorine, taken in conjunction with the less marked retention of nitrogen referred to in the last section, may possibly be attributed partially to the increase in cancer tissue and to œdema, but also probably to a considerable extent to its association with products of suboxidation retained in the system.

The ammonia secretion in the first period was very large, reaching a total of 4.1 grams as against 1.75 in the second period, showing a marked improvement in the presence of large quantities of chloride.

The removal of uric acid and other bodies capable of reducing permanganate was, on the other hand, found to be larger in the second period than during the first. Whilst in the first period, with a limited chlorine diet and in spite of the large volume of the urine, only 1.26 grams of uric acid were removed, in the second period 1.6 grams were excreted with the urine.

Phosphates, which were absolutely constant in the diet, were also found to be practically constant in the urine and fæces, showing an average from 2 to 2.2 grams H_3PO_4 per diem.

An analysis of the blood showed a freezing point .57° and sodium chloride content .47 per cent, both of which are practically normal.

To summarize the results in this case it may be said that in the absence of toxic symptoms a practical equilibrium of nitrogen is established and a small tendency to retain chlorides exhibited.

CASE IV.

Mr. T.; age 66; Scotch; married.—Patient gives no family history of cancer or of tuberculosis. He has not had specific disease but has been intemperate. He gives an indefinite history of ten years' illness, referred particularly to the abdomen and kidneys. For the last few months he has had frequent urination, and the specimen contained varying quantities of blood. In the past year he has lost weight progressively and for the past five months has been confined to bed.

Patient died January 17, 1903, at the Buffalo General Hospital, and autopsy revealed the following conditions: Very marked emaciation. On opening the chest the right pleural cavity showed several large tumors the size of a plum, with cancerous disease of several ribs on the same side. The lung tissue was quite normal, and the bronchial glands were not diseased. In the abdominal cavity the lymph glands beneath the peritoneum were enlarged and hard. The stomach was free from cancerous change. The liver showed in the upper portion of the right lobe a slight metastatic carcinomatous growth. The mesenteric glands were not noticeably changed. The left kidney was large and soft while the ight kidney was the seat of a large primary, carcinomatous tumor.

In this case a balance of the nitrogen and chlorine of the food and excreta was carried out for the nine days immediately preceding the death of the patient (see Table IV). The diet consisted exclusively of one or two eggnogs daily, the exact nitrogen and chlorine content of which was known. For two days a slight increase in the salt of the food was not associated with any considerable increase in that of the urine, but patient showed more marked œdema and became extremely cachectic. An examination of the table will show that whilst a steady loss of nitrogen was exhibited for the entire period, there was a strong tendency to retain chlorides in the system, and, as stated above, a slight increase in the chlorides of the food did not lead to greatly increased excretions and simply tended to accentuate the œdema of the patient. The uric acid nitrogen in comparison with the total nitrogen of the urine was high, varying from 1.7 to 2 per cent. The ammonia was above the normal from 5 to 8 per cent. In this case, in which the functions of the kidneys were materially interfered with, we have a marked illustration of the toxic symptoms associated with a considerable loss of nitrogen and a tendency to retain chlorides.

TABLE IV.

	Nitrogen of food.	Nitrogen of excreta.	Chlorine of food as *Na Cl.*	Chlorine of excreta as *Na Cl.*
8–9	5.1	8.3	2.0	.32
9–10	5.1	8.6	2.0	.32
10–11	5.1	10.5	2.0	.62
11–12	2.6	3.9	1.0	.08
12–13	5.1	5.4	2.0	.33
13–14	5.1	6.3	3.6	.55
14–15	5.1	6.8	3.6	.82
15–16	5.1	7.1	2.0	.45
16–17	2.6	4.5	1.0	.10
Total	40.9	61.4	19.2	3.59

CASE V.

Mr. C.; age 75; weight 65 kilo.—Sarcoma of skin and connective tissue. Patient presents a nodular discolored area in right epigastric region having a maximum diameter of six inches. Numerous tumor nodules, some of them as large as a walnut, apparently seated in the skin and the subcutaneous tissue, in the discolored area. Patient refused to submit to operation and left the hospital at the completion of the series of experiments. Whilst exhibiting toxic symptoms, his appetite was normal and nutrition apparently unaffected. For the twelve days that he remained in the hospital he received a diet containing approximately 11½ grams nitrogen and 8 grams sodium chloride per diem.

The analyses of urine and fæces were conducted with as great accuracy as those previously referred to, but in the diet a possible source of error is introduced, since analyses were only carried out on two days in the course of the experiment, giving 11.3 and 11.6 grams nitrogen and 8 and 8.1 grams sodium chloride respectively; on the remaining days the food being weighed to correspond as nearly as possible to the same amount. Our principal reason for including this case is the evidence which it affords of both a nitrogen and chlorine balance, even in the presence of a tumor which has reached considerable dimensions and in spite of marked toxic symptoms, provided the digestive organs are unaffected. A slight gain of nitrogen was apparently effected during each period, and the chlorides determined for the second six days showed a similar close relationship (see Table V); all of which corresponds with the body weight of the patient, which remained constant throughout the experiments. It is also nteresting to note that in this case the uric acid nitrogen was averagng 1.4 per cent of the total nitrogen.

TABLE V.

)ay.	Total nitrogen of food.	NITROGEN OF		Sodium chloride in food.	SODIUM CHLORIDE IN	
		Urine.	Fæces.		Urine.	Fæces.
1..	Approximately	9.4	for six days.	....	....	....
2..		7.3		....	....	....
3..		9.4		....	....	....
4..		10.7		....	....	....
5..		8.1		....	....	....
6..		10.1		....	....	....
	69.0 to 71.0	55.0	10.50			
		65.5				
7..	Approximately	10.1	for six days.	Approximately.	8.2	Only traces; disregarded.
8..		9.0			6.7	
9..		9.3			8.1	
0..		9.1			8.2	
1..		9.8			8.2	
2..		10.5			9.5	
	69.0 to 71.0	57.8	9.7	46.0 to 48.0		
		67.5			48.9	

The analysis of the blood showed 2.5 per cent nitrogen and .48 per cent NaCl.

To summarize results in this case it may be said that, in spite of the age of the patient and the development of the tumor, practically no effect has been exerted on his nutrition, which is fully up to the normal standard of a healthy individual of his age.

Case VI.

Mrs. N.; age 58; married; weight 44 kilo.—No family history of cancer or tuberculosis. The patient can give no definite account of the duration of the disease except that it existed about a year, and that after it was first noticed in the vulva it was of considerable size and progressed very rapidly, particularly so in the past three months. On examination there is found extensive tumefaction of the right labium, nodular, hard and ulcerating on inner surface. There are also some nodules on the left side but not large. The entire vagina and cervix are involved in the tumor mass. There is much necrotic tissue and a dark, very offensive discharge. The growth on the right side is about the size of a man's fist. There are metastases in the immediate neighborhood of the right groin and perineum, also quite a large nodule in the right buttock. Patient is very much emaciated, feeble, cachectic and much of the time unable to take sufficient food, this last condition being aggravated by her mental state of depression. She has much pain, so much so that anodynes are required to give her rest and sleep.

In this case analyses were made on the 24-hour specimens of the urine for 45 days. For 24 days estimations of nitrogen and chlorine in the food, in the urine and the fæces were also carried out, but owing to the difficulty of properly controlling the patient only one period of six days can be looked upon as reliable.

1. *Nitrogen and chlorine equilibrium.*—For the first twelve days complete failure to effect an approximate balance of the nitrogen and chlorine led to the discovery that the patient was deliberately throwing away a portion of the urine and fæces, so that results were obtained similar to those of Moraczewski, showing a retention of nitrogen and chlorine, at the same time an appreciable loss in body weight being observed. Such results being obviously incorrect, for a period of six days patient was carefully watched and a fresh series of experiments showed a nitrogen consumption of 48.6 grams and a nitrogen loss of 54.2 grams.

At the same time 36.1 grams sodium chloride had been consumed with the diet and 34.2 grams excreted in the urine. Even these figures failed to afford a satisfactory balance, a loss of 5.6 grams nitrogen and a gain of 1.9 grams sodium chloride being scarcely comparable with the actual loss in body weight during this period of .75 kilo. One possible source of error, the secretions of the skin, will be considered at a later stage, but it must be concluded that cases of this type are not suitable for metabolism experiments, owing to the difficulty of controlling the loss which takes place continually from the surface of the ulcerating tumor. On the other hand, the 24-hour analyses may be made use of to compare the relative proportions of nitrogen, uric acid, ammonia, chlorine and phosphorus. The uric acid nitrogen was found as an average of the 45 days to be 2.1 per cent of the total nitrogen; the ammonia nitrogen 12.5 per cent; phosphorus 7.4 per cent and the chlorine 40 per cent of the total nitrogen.

[*]Case VII.

P. G.; age 59; Polish.—Committed to Erie County Hospital March 14, 1904. Was found to be much emaciated; complained of inability to swallow solid foods but was able to take fluids. Examination with œsophageal bougies showed that there was some dilatation of the œsophagus and an obstruction at the cardiac opening. After several attempts, however, a moderately large bougie was passed into the stomach. He was kept under observation, and on May 21st a gastrostomy, typical Witzel, was performed. He was fed through the tube for about a fortnight, at which time the irritation in the œsophagus had so subsided that he was again able to swallow semisolid food with ease and some solid food. His condition steadily improved; he gained in weight and on June 15th tube was removed and shortly afterward the opening closed spontaneously. Patient's ability to swallow semisolid food and at times solid food remained unchanged until September, when he contracted lobar pneumonia, of which he died on October 5th.

The condition of the œsophagus on autopsy was shown to be slightly enlarged; there was no definite area of erosion or permanent stricture of the cardiac opening. The mucous membrane of the œsophagus was somewhat injected. The anatomical findings confirmed the clinical diagnosis of cardio-spasmus.

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[*] We are indebted to Dr. Gaylord of this Laboratory for above history and autopsy notes.

In view of the possibility that this was a case of cancer of the œsophagus a series of nitrogen and chloride determinations was carried out shortly after the operation had been performed. The patient's diet throughout this and subsequent experiments consisted of milk, with the addition of definite quantities of plantose (a vegetable albumen) and salt, meat broth and egg-nogs, which greatly facilitated the determinations of nitrogen and chlorine. The first series, *a,* as will be seen from the table, shows a distinct loss of nitrogen and fair equilibrium of chlorides. A heavy nitrogen loss at this stage must, however, be attributed to the shock of the operation and to the toxic effects of an infection which developed at the point at which the tube was introduced. A subsequent series, *b,* of nitrogen determinations carried out six weeks after the operation and previous to the development of pneumonia shows a very fair maintenance of nitrogen equilibrium. With regard to the chloride metabolism, it is interesting to note in series *a* that, whilst a heavy loss of nitrogen had taken place, probably owing to toxic influences, a very fair chloride equilibrium was maintained. There was no tendency to œdema, and the toxic influences referred to above apparently failed to exert such an effect upon the kidneys as to lead to a retention of sodium chloride.

TABLE VI.

Series a.

DAY.	Nitrogen of food.	Nitrogen of excreta.	NaCl of food.	NaCl of excreta.
1	12.5	15.7	5.5	4.8
2	11.4	18.6	4.2	4.3
3	12.3	16.6	6.3	6.4
4	12.8	17.7	4.3	5.7
5	12.6	16.9	6.8	6.9

Series b.

DAY.	Nitrogen of food.	Nitrogen of excreta.
1	14.0	14.3
2	13.6	10.4
3	13.4	15.9
4	13.9	13.3

Estimations of uric acid and ammonia as compared with the total nitrogen of the urine over an extended period showed very low uric acid and fairly high ammonia results, the uric acid nitrogen averaging less than .8 per cent of the total nitrogen, the ammonia nitrogen, especially during the toxic period, being as high as 10 to 15 per cent of the total nitrogen.

* CASE VIII.

L. L. ; age 44 ; weight 150 pounds ; married.—Born in the United States of German parentage. Retired butcher. No family history of cancer or tuberculosis on either side. No personal history of syphilis or intemperance. Patient enjoyed ordinarily good health until the year 1897, when he states that he had an illness of two months duration characterized by much pain in the region of liver and marked jaundice, indigestion and fever. No evidence of gall stones. In April, 1904, had pain in the right hypochondria, but no vomiting, belching or other gastric symptoms. From July 1st experienced considerable difficulty in swallowing solids, and some with regard to liquids ; belched gas and had small regurgitation of food with considerable mucus, but without noticeable color, odor or taste. At this time he suffered from sharp epigastric pain, which was almost continuous, and at the same time from constipation. A physical examination revealed nothing abnormal as to liver, stomach, heart, kidneys or appendix. On July 26th the œsophagus was sounded. The largest sized bulbs entered easily to a depth of 29 cm. from the incisors. Size 26 (French) entered to a depth of 30 to 31 cm., varying according to force employed but could not be inserted lower than 31 cm. Size 22 passed the stricture with some difficulty and it was thus determined that the lower œsophagus was sufficiently large to accommodate this bulb below a point 33 cm. from the incisors. On August 8th an X-ray examination was made, but revealed nothing as to the cause of stricture. On October 11th an attempt was made under ether to inspect the stricture by means of the speculum, but on account of a stoop of the shoulders it was not possible to straighten the spinal column sufficiently. At various times œsophageal bulbs were passed corroborating the results of the first examination and showing neither increase nor abatement of the stricture. No blood nor tissue was at any time found, the microscopical examination revealing nothing but epithelial scales and food remnants. Patient had lost weight considerably previous to the 26th of July, on which date he weighed 150 pounds. The experiments in metabolism referred to later were carried out between the first of August and the middle of October, at which time he weighed 158 pounds, having gained eight pounds in the course of the experiments. A careful investigation reveals no history of tuberculosis, syphilis or traumatism including corrosion, and since cardio-spasmus can be ruled out, reasoning by exclusion the case is probably one of cancer of the œsophagus.

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* We are indebted to Dr. A. L. Benedict of this city for the above history of the case.

From the above history it will be seen that, as the nature of the obstruction of the œsophagus is not known, it can not be said positively whether a tumor is present or not. A series of nitrogen and chlorine equilibrium experiments were carried out in conjunction with work on the nutrition of patient and the results over an extended period are included, so that they may be compared with those obtained on the cases known positively to be cancer, Nos. II, III, IV, V and VI, with the normal individual case (I) and the case of cardio-spasmus (VII). By the addition of plantose (a pure vegetable albumen) to milk, in varying proportions, it was found possible to convey sufficient food to the stomach of the patient to bring about a gain of 6 kilo in weight in the course of three months. The first series of experiments was conducted with a view to determine the amount of plantose which could most suitably be added to milk and eggnogs in order to give the requisite amount of proteid to maintain the nitrogen equilibrium between the food and excreta. For this period patient's diet consisted of an eggnog once daily, and 250 c.c. of milk with the addition of 20 grams of plantose and 3 grams of salt, three times daily, with milk in addition according to the desire of the patient.

From Table VII, in which the results of this first period are recorded, it will be seen that the loss of nitrogen, averaging 14.6, is only slightly in excess of the nitrogen of the food, averaging 14.4, quantities fairly in accord with the weight of the patient. During this period the chloride equilibrium was not determined with any accuracy, the results indicating a slight tendency to salt retention.

During the second period the nitrogen of the food was diminished to an average of 10.9 grams daily, with the result that a distinct loss of nitrogen was suffered by the system, averaging 1.7 grams daily. The total nitrogen secreted in the urine and fæces, averaging 12.6 grams, is not, however, indicative of toxic influences leading to abnormal destruction of body proteids, a fact which is further confirmed by a relatively low uric acid nitrogen. During this period the chlorides were maintained at a fairly high level, no very considerable differences being observed, an average of .9 grams sodium chloride being retained in the system daily.

TABLE VII.

Period 1.

Nitrogen of food.	Nitrogen of excreta.
13.0	14.6
13.0	13.1
14.2	15.2
15.5	14.5
15.5	14.4
15.5	16.2

Period 2.

Nitrogen of food.	Nitrogen of excreta.	NaCl of food.	NaCl of excreta.
11.4	13.3	12.3	11.2
10.7	12.3	11.7	12.4
10.7	12.3	11.7	9.4

Period 3.

Nitrogen of food.	Nitrogen of excreta.	NaCl of food.	NaCl of excreta.
16.8	14.8	11.7	9.5
16.8	17.6	11.7	11.9
16.9	15.6	11.7	10.9
17.0	13.6		
17.1	14.6		

In the subsequent Period 3 the amount of proteid in the food was materially increased to an average of 16.8 to 16.9 grams daily, with the result that a distinct retention of nitrogen was to be observed. For the first three days of this period the nitrogen intake exceeded the loss by 2.5 grams and for the two following days by as much as 5.9 grams, but it was subsequently found possible to diminish the nitrogen content of the food to 15.5 to 16 grams, at which figure patient gained weight and showed a marked improvement, conditions which are seldom associated with the development of a malignant tumor. For the first three days of this period the chlorides were excreted in a perfectly normal manner, as much as 11.7 grams in the diet being accompanied by a loss of 11.1 in the urine. A determination of the uric acid nitrogen showed a uniform content of .9 to 1.0 per cent of total nitrogen throughout the entire period, a figure which is below that generally obtained by ourselves and other investigators in cases suffering from a malignant tumor. This evidence of the absence of toxic influences would not, however, in itself justify us in deciding against the probability of the presence of a tumor.

At this stage reference must be made to a source of error which has been ignored by practically all those who have devoted attention to work on metabolism. The secretions of the skin, whilst possessed of a very small solid residue in normal healthy individuals, may reach considerable dimensions under pathological conditions which necessitate a performance by the skin of a portion of the kidney function. The meager analytical data bearing on this subject to be found in the literature would indicate a normal loss of .1 to .2 grams nitrogen and .2 to .3 grams sodium chloride per diem, but records are to be found of quantities as large as .5 grams nitrogen and 1 to 1.5 grams sodium chloride having been excreted, under pathological conditions, by the skin. Whilst the sources of experimental error in such work are so enormous as to preclude the possibility of obtaining strictly accurate figures, it is perfectly conceivable that even larger quantities than the above might be removed in the space of 24 hours. In any case it appears probable that the loss of sodium chloride by this means is from two to three times that of nitrogen, and consequently would afford a partial explanation of the fact observed in the majority of our own cases, as well as in those of most previous investigators, that the ratio of sodium chloride to nitrogen is lower in the excreta than in the food consumed. Assuming a minimum loss of .1 grams nitrogen and .2 grams sodium chloride and a maximum of .5 nitrogen and 1 gram sodium chloride, it will be seen at a glance that whilst the huge discrepancies recorded by Moraczewski of 8 or 9 grams difference between the chlorides of the food and those of the urine and fæces would not be materially affected, such a quantity would go far to supply the deficiency of chlorides in the urine observed in certain of our cases.

This source of error may well be advanced as an explanation of the slight discrepancies frequently observed in the balances of body weight, nitrogen and chlorine intake and output, and the osmotic tension of sodium chloride retained in the system, which should involve a retention of water and consequently an increase of body weight. For example, in Case II the observed loss of 77.5 grams nitrogen and a retention of 25 grams chlorine, or 41 grams sodium chloride in a period of eight weeks, would be changed to a loss of 80.7 grams nitrogen and a retention of only 18.7 grams sodium chloride, assuming that a daily loss of .2 grams nitrogen and .4 grams sodium chloride had been suffered by the system through the skin secretions. These figures would correspond far more closely with the variations in weight of the patient, which show from first to last a gain of about half a kilo, presumably attributable to œdema. Such an explanation would remove any necessity for assuming an extraordinarily increased concentration of sodium chloride or diminished dissociation of that substance into ions in the body fluids and cells, the only other conceivable explanation of the discrepancies observed. In other cases in which the daily retention of sodium chloride averages from .5 to 1 gram it will be seen that the loss of this substance by the skin may produce an appreciable difference; it must, however, be said that in those cases in which the salt consumption is small any great loss by way of the skin is improbable.

The blood.—In view of the evidence afforded by some of the above cases that in the last stages of cancer a heavy loss of nitrogen resulting from the destruction of body tissue may be counterbalanced in its effect upon the body weight by a retention of chlorides leading to the formation of œdema, it seemed a matter of considerable importance to carry out analyses on the blood and body fluids of such cases to observe deviations from the normal. Whenever sufficiently large quantities of blood were procurable, estimations were made of the nitrogen, chlorine, phosphorus, the lowering of the freezing point and titrations to the end points of a series of indicators. Blood counts were carried out from time to time, but only as required for clinical purposes, since the very thorough work of Kronkiewicz on this subject, showing variations in reds from 1,800,000 to 6,000,000 and whites from 4,000 to 20,000, rendered further work in this direction superfluous. It is very much to be regretted that, owing to the risk of endangering the life of the patient, we were able to obtain blood in sufficient quantities in only one or two cases in which patient exhibited marked cachectic symptoms, and consequently it was seldom possible to perform all the analyses referred to above in addition to the tests for hæmolysins, etc., referred to previously in this report. We have, however, a series of analyses on cases in which tumors had reached considerable proportions and discrepancies in the nitrogen and chlorine equilibrium had already been remarked, and the results can scarcely be said to deviate from the normal to the extent that one would be led to expect from the literature. The nitrogen, chlorine and phosphorus results varied considerably, showing a slight tendency to diminished nitrogen and increased chlorine (see Table VIII), but scarcely to the same extent as did those of Moraczewski. Titrations carried out on the serum after separation from the clot showed with one exception results very little below the normal in alkalinity. N/50 acid was employed, and from the tabulated results in which the amount of acid required to neutralize 1 c.c. of serum to given end points are recorded, a comparison may be drawn between our cases and those of Rigler. This investigator observed a fall in the alkalinity, both of the blood as a whole and of the serum, in a series of diseases, reaching an acute stage shortly before death, and in case of recovery once more rising to the normal. Using lakmoid as an indicator, he found 1.8 to 2.2 c.c. n/50 acid was required to titrate 1 c.c. of normal blood serum, whilst in acute diseased conditions this figure sometimes fell as low as 1.2 and even 1.1. Our records, whilst slightly below the normal, never reached these figures. (See Table IX.)

Engelmann, in a paper on the freezing point of the blood of cancer patients, finds variations from .56, the normal, to .65 in inoperable carcinoma ventriculi. *We have at present examined only six cases, five of which lie within the normal limits from .56 to .60, one being slightly over the latter figure. We are not therefore able to bring forward any results in support of the generally accepted view that the osmotic pressure of the blood serum of advanced cancer cases is necessarily above the normal, but are rather inclined to consider such variations as attributable to deficient heart and kidney function. It is, however, our intention to deal with the question of blood analysis more thoroughly at a later date, making use of a series of cancer animals. Since, however, gross analyses are of little value in dealing with the disturbances in osmotic equilibrium of cancer-cells, owing to the

TABLE VIII.

Analyses of Blood of Cancer Cases.

	Nitrogen.	Sodium chloride.	Phosphorus.
Cancer of the breast	2.13	.48	.03
Cancer of the stomach	1.84	.47	.08
Cancer of the liver	2.24	.45	.035
Cancer of the stomach	2.30	.49	.04

TABLE IX.

Blood Serum (Cancer Cases).

	Phenolphthalein.	Rosolic acid.	Lacmoid paper.
Cancer of liver	—.2	.7	1.71
Cancer of liver and stomach	—.4	.8	1.8
Cancer of stomach	....	.58	1.66
Cancer of intestines	....	....	2.2

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*Analyses on materials obtained at autopsies (see p. 86) are, of course, excluded from these results.

great delicacy of such variations, we are devoting attention rather to the osmotic equilibrium of the individual cell and to the interchange of salt ions capable of passing through the cell walls in the hope of thereby throwing some light on the questions as to whether or no specific toxic influences exerted on the protoplasm of the cells are bringing about changes in their viscosity and permeability.

Whilst it is out of the question to summarize in any satisfactory manner the numerous isolated details brought out in studies in metabolism, and the average of a large number of results gives no idea whatever of the course actually taken in the establishment of any state of equilibrium, we have included a table showing the averages of the nitrogen and chlorides of the food and excreta for considerable periods of time in a series of cancer cases compared with the normal. Such a table is necessarily influenced to a certain extent by experiments carried out (in the course of the period, an average of which is presented) with a view to observing the effect of nitrogen or chlorine starvation or the administering of an excess of either of these substances. It will, however, enable us to recognize any extensive loss or gain of nitrogen or chlorine suffered by the system as the disease develops.

Case I, a normal individual, was submitted to partial proteid starvation and at the same time received widely varying quantities of sodium chloride. As might be expected a slight nitrogen loss was suffered by the body, whilst a marked tendency to the establishment of chloride equilibrium was exhibited.

In Cases II and IV, both carcinoma, in which cachexia had intervened, and the patients were suffering from partial proteid starvation and succumbed to the disease at the completion of the experiments, as well as in other cases records of which are incomplete, a marked loss of nitrogen and retention of chlorides was to be observed. At the same time it must be noted that the excretion of 6.7 grams nitrogen daily is very little above that of the normal individual in a state of starvation, and it does not indicate that the toxic effects of the tumor have led to an excessive proteid decomposition in the body.

Case III is remarkable when it is remembered that the patient's stomach and liver were riddled with carcinoma, and yet so slight an interference exerted upon the nutrition that a retention of nitrogen as well as sodium chloride in the course of our experiments was observed. One source of error unfortunately neglected was the loss of nitrogen and chlorine, which took place from the mouth and throat on expectorating after coughing and the employment of a gargle. It was our intention to repeat the experiments in question, but unfortunately the rapid decline in the state of the patient, followed very shortly by death, excluded the possibility of further investigation in this case.

Case V shows how little effect a rapidly growing sarcoma may exert upon the nutrition, and the nitrogen and chlorine equilibrium of a patient, a practical balance of these bodies being obtained for a space of twelve days.

Case VI, a markedly emaciated and cachectic case of epithelioma of the uterus, showed in the course of our experiments a loss of nitrogen and a retention of chlorides. The differences observed were very slight, a fact which may possibly be attributed to experimental errors resulting from the difficulty of controlling a patient of this type.

In Case VII (cardio-spasmus) as the result of an operation and subsequent infection, the loss of nitrogen in the first period is very considerable, whilst a fair equilibrium of chlorides is maintained. In the subsequent period, patient having recovered from the infection, the nitrogen balance is about normal.

In the last case recorded in the tables, suffering from stricture of the œsophagus, the cause of which is unknown, whilst a nitrogen equilibrium is established on a daily consumption of proteid containing 14 grams nitrogen, a smaller quantity in the diet leads to a loss on the part of the body and an increased quantity to retention of nitrogen. There is possibly a slight tendency to retain chlorides in this case.

We have in Table XI included some of the cases investigated by Müller and Braunstein, from which it will be seen that whilst Müller records a daily loss of nitrogen exceeding 5 or 6 grams in six cases out of eight, Braunstein, whilst obtaining a fair equilibrium in the majority of cases, records one in which as much as 10 grams nitrogen was lost daily through the urine and fæces in excess of that present in the diet. The nitrogen results obtained by the latter investigator correspond fairly well with those which we have obtained; but whilst we have noted a distinct retention of sodium chloride in all cachectic carcinoma cases, he finds a loss in four cases out of five.

TABLE X.

CASE.		Nitrogen of food.	Nitrogen of excreta.	Sodium chloride of food.	Sodium chloride of excreta.
I Normal..................	a	13.0	13.4	11.4	11.9
	b	11.6	13.2	13.1	13.5
II Carcinoma of stomach and pancreas................	a	6.5	6.5	1.85	1.8
	b	5.5	6.7	1.6	.9
	c	4.3	6.7	1.9	.45
III Carcinoma of œsophagus involving stomach and liver....................		9.4	8.5	5.4	4.45
IV Carcinoma of kidney..........		4.5	6.8	2.1	.4
V Sarcoma of skin and connective tissue...........		11.5	11.1	7.8	8.1
VI Epithelioma of uterus, etc....		8.1	9.0	6.0	5.7
VII Cardio-spasmus	a	12.3	17.1	5.4	5.6
	b	13.7	13.5		
VIII Œsophageal stricture (cause to be determined. See page 97)...........	a	14.4	14.7		
	b	10.7	12.6	11.9	11.0
	c	16.9	15.2	11.7	10.8

TABLE XI.

CASE.	Nitrogen of food.	Nitrogen of excreta.	Sodium chloride of food.	Sodium chloride of excreta.	
V Carcinoma ventriculi ..	3.9	11.4	......	......	Müller
VI Carcinoma ventriculi ..	3.45	12.0	.....	......	“
VII Carcinoma ventriculi ..	2.45	9.1	......	......	“
VIII Carcinoma pancreatis..	1.7	6.0	......	......	“
IX Carcinoma mammæ ...	9.1	10.4	......	......	“
I Carcinoma œsophagi ..	7.7	6.5	5.1	5.6	Braunstein
VI Carcinoma œsophagi ..	7.7	7.8	7.0	7.8	“
VIII Carcinoma œsophagi...	7.2	10.3	4.4	3.7	“
IX Carcinoma ventriculi ..	8.8	10.6	7.3	8.5	“
X Carcinoma œsophagi ..	4.2	14.9	.690	.794	“

Moraczewski's results are exactly the reverse of those obtained by Müller and Braunstein. In one case of carcinoma of the uterus he records a retention of 43 grams chlorine and 52 grams nitrogen, the patient merely gaining one kilo in weight. In a case of carcinoma of the ventricle he observed a chlorine retention of 82 grams with a slightly larger variation in weight. In Case VI, carcinoma of the ventricle, with one kilo increase in weight in the course of eight days, a retention of 75 grams nitrogen and 45 grams chlorine was observed. Such proportions are obviously impossible. An increase of merely one kilo in body weight could not conceivably be associated with a retention of quantities of chlorides and nitrogen as large as those recorded in the first and last examples. One must, however, conclude from his publications that he considers his cases afford evidence of a strong chlorine and nitrogen retention, whilst Müller is convinced that the loss of nitrogen is in the majority of cases extremely heavy. Braunstein finds in one case only out of ten any considerable loss of nitrogen, in the remaining cases a slight loss in body weight was observed. Our results differ considerably from either those of Müller or Moraczewski and are more in accord with those of Braunstein. We are inclined to believe that in all cachectic carcinoma cases a loss of nitrogen and a gain of chlorine may be looked for, especially where there is any tendency to œdema, but we have not in our experience met with any such huge differences in the intake and output either of nitrogen or chlorine as are to be found in the tables of either of the three investigators referred to above.

It must be confessed that none of our own cases affords an absolutely satisfactory balance of the various factors referred to in the introduction, change of body weight, loss and gain of nitrogen and chlorine, increase in weight of tumor, loss of tissues and production of œdema. It may, however, safely be said that when allowance has been made for a possible slight loss of nitrogen and chlorine by way of the skin and mouth, and for the other sources of experimental error, all of which tend in the same direction, viz: to an apparent diminution o the nitrogen and chlorine secretions, a fair balance is to b struck in all cases in the metabolic equilibrium. Whilst ther is still a margin of chloride retention which would lead one t suppose that the excess chlorine held in the body can not b accounted for exclusively on the basis of increased fluid content still the differences are not nearly as large as in Moraczewski' cases, and a slightly increased concentration of sodium chloride i the tissues associated with a decreased dissociation of that substanc so as not unduly to disturb the osmotic equilibrium, might afford th necessary explanation. Unfortunately for this theory, we hav not been able to obtain chemical evidence of any marked change in this direction in the blood and tissues; but it must be remembered that existing methods of handling such a problem are of necessity crude and the changes to be investigated extremely delicate

As regards the effect of increasing the quantity of chloride in the diet and the effect produced by this means on the œdem of the patient, it must be said that whilst normal individual show a tendency to establish a chloride equilibrium with ver great readiness in the space of from 24 to 48 hours, being capable of consuming large quantities of chlorides above the norma without any marked changes in the blood and tissue, cachecti cancer cases, especially those in which the kidneys are deficien in their activity, are incapable of reacting even to a small increas in the amount of salt, marked œdema resulting from any suc increase, which only disappears on once more reducing th amount present in the food. For details regarding the effec on the body weight and the secretion of metabolic product brought about by variations in the amount of chlorides in th diet, reference must be made to the analytical results recorde under each case.

The great significance of the role played by soluble chloride in the processes of oxidation and elimination of waste product can not be overlooked, and we are not inclined to agree wit Braunstein in assuming that the increase or decrease in the secretion of salt is simply a question of the amount of water retaine in the system with which a certain amount of salt would normally be associated. We are inclined rather to look upon the water as being retained in conjunction with the salt, which in its turn is possibly prevented from proper diffusion by reduction in the permeability of membranes and by its possible association with products of metabolism. For the relative proportions of ammonia, uric acid and other nitrogenous constituents of the urine, phosphates, etc., reference must be made to the individual cases, or to a subsequent article in this report, in which this question is dealt with more in detail. Whilst increased secretions of uric acid and ammonia in proportion to the total nitrogen are frequently observed and assumed to indicate respectively disintegration of tissue rich in nuclein and faulty oxidation, it must be borne in mind that variations in the nature of the food consumed can bring about very marked changes in the proportions of these substances, as may be seen in Cases VII and VIII.

In conclusion it must be said that whilst work of this nature affords considerable evidence of the presence in the system of toxic bodies capable of seriously interfering with the nutrition of the patient, there is no positive evidence that we have to deal in cancer with toxins of a specific type, such as are invariably associated with the course of development of diseases caused by bacteria and other recognized parasites. The presence of nitrogenous suboxidation products resulting from some interference with the metabolism of the cells may well exert an accumulative effect, and as the disease progresses cause all the symptoms with which we are familiar in cancer cachexia.

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CHEMICAL EXAMINATION OF THE URINE AS A MEANS OF DIAGNOSING THE POSITION OF A TUMOR AND OBSERVING THE COURSE OF ITS DEVELOPMENT.

By G. H. A. Clowes, Ph. D.

A paper dealing with this subject and summarizing the results obtained by previous investigators was published by Blumenthal in the report of the Krebsforschung Komité, 1902. The conclusions arrived at by this author possess a considerable amount of value, especially in view of the difficulties that are associated with a thorough investigation of the urine. As the result of analyses carried out on a series of cancer cases we are in a position to confirm for the most part the results recorded in his paper, but in certain cases variations worthy of note have been observed. For the literature reference must be made to the original article. In a previous portion of this report the question of retention of chlorides in the system has been completely dealt with, and it is therefore only necessary to repeat the statement that in cases of advanced carcinoma, where accurate analyses have been carried out on the diet and urine, a considerable loss of nitrogen and retention of chlorides has been observed. It has been suggested by Schöpp that the chlorine apparently retained in the system is really removed from the surface of ulcerating tumors. This explanation was not, however, applicable in any of the cases under observation, with the exception of Mrs. H., in which it received due consideration. That the total chlorides in the urine are so considerably diminished as to be present in proportions as low as one per cent to one-half per cent of the total nitrogen, or even to entirely disappear, whilst the proportion of chlorine to nitrogen in the fo is still as 1 to 3, is a matter of daily observation, but it mu be borne in mind that the extremely low secretion of chlorid observed by early investigators might frequently be attribut to the diminished proportions of chlorides contained in a mi or fluid diet as compared with a solid diet. In the cases whic have been under observation in this Laboratory, so long as t chlorides in the diet were maintained at a normal level of n less than 3 or 4 grams chlorine per diem, and so long as t individual was capable of assimilating solid food, the proportio of chlorine excreted in comparison with that of nitrogen seldo fell below 50 per cent, and it was only when the fatal termin tion of the disease was approaching that such figures as 2 p cent, 1 per cent and even less were recorded. In fact, the resul correspond fairly closely with those observed by Müller, etc., o starving individuals. The explanation offered by this auth that the organ proteids which are relatively poor in chlorid were undergoing decomposition in place of the circulating pr teids needs further support before it can be accepted.

In six cases in which exact determinations of phosphoric ac in comparison with nitrogen were carried out for a considerab period the amounts of phosphoric in proportion to nitrogen we found to be 6.8, 6.8, 7.2, 7.5, 7.7 and 7.8 per cent, giving an avera of 7.3, which corresponds fairly closely with the highest result 7.3, obtained by Müller, and is slightly in excess of the norm As regards various nitrogen compounds, we have already dea with the question of the total nitrogen excreted in compariso with the amount assimilated, and have found that when d allowance is made for all possible sources of waste the amou lost is usually slight and sometimes considerably in excess that present in the food. The proportions of the various co stituents differ widely. Whilst normal urine may be said contain from 90 to 95 per cent of its nitrogen as urea, about to 1.5 per cent as uric acid, 1.5 to 4 per cent as ammonia a the residue as extractive and amido acid nitrogen, similar pr portions have been found to obtain in the very early stages development of those cases of cancer in which the tumor produced no direct effect upon the metabolism and did not interfere with the nutrition. In all cases in which a marked cachectic condition had set in and where the nutrition of a patient was seriously impaired, either by toxic effects or the mechanical development of the tumor, results such as the following were to be observed: Urea nitrogen from 85 to 90 per cent; ammonia nitrogen from 6 to 12 and even 15 per cent; uric acid nitrogen from 1.8 to 2 and even 3 per cent, as compared with .9, 1.0, to 1.3 in other cases. It can not be too strongly emphasized that such results were only obtained in those cases in which inanition, accompanied by the pathological destruction of proteids, had reached an advanced stage. Also it must be remembered that the nature of the diet has a great effect on the uric acid secretion.

Halpern, in fractionating the nitrogenous constituents of pathological urines, presents four cases of cancer, two of them being carcinoma of the stomach with metastases in the liver, one carcinoma in the region of the porta hepatis and the fourth a carcinoma cardiæ. In the first case, carcinoma of the stomach with metastases in the liver, a low urea accompanied by high purin body nitrogen (including uric acid) high ammonia and normal extractive nitrogen were recorded. In the remaining hree cases the proportions of the various constituents were not ar removed from the normal, the extractive nitrogen being, however, rather high but not in any sense comparable with the high esults obtained by Töpfer, Setti and Gumlich for extractive nitrogen, reaching figures from 10 to 23 per cent. We have not n the course of our work met with any such great deviations rom the normal as are recorded by these authors, and in one ase, carcinoma of the stomach with metastases in the liver, in which o very serious interference with the nutrition was to be observed, ractically normal results were recorded for ammonia and uric acid.*

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*Experiments on a series of cases fractionating carefully the nitrogeous constituents of the urine are being carried out in the Laboratory at he present time and will be reported upon when completed.

We have been unsuccessful in an attempt to confirm the observation of Griffiths, who claims to have isolated a specific alkaloid having the formula $C_8H_5NO_3$ from cancer urine by extracting with ether in urine previously evaporated in vacuo and treated with alkali. Our experiments resulted in the separation of kreatinine, traces of xanthine bases and other normal urinary constituents, but not of any compound which could be looked upon as specifically associated with cancer. The observation made by Bouchard that the urine of cachectic cancer patients is more toxic than the normal was confirmed, but this may well be attributed to the larger proportion of normal sub-oxidation products; and, as stated above, the search for any specific ptomaine has been unsuccessful. A large proportion of cancer cases appear to succumb rather to starvation than to any toxic effects exerted directly by the tumor in its development. Any interference with the metabolism of the patient naturally leads to the production of toxic products which induce cachectic symptoms, and, as Müller observed, the noticeable difference between the loss of nitrogen suffered by the normal starving individual and one dying of starvation as the result of interference of a tumor is a slightly more rapid destruction of body proteid and elimination of nitrogen in excess of food consumed per kilo of body weight in the latter case over the former.

The secretion of phenol and indican in the urine in cases of stomach and intestinal cancer frequently reaches from two to four times the normal, but we have seen one case in which a stomach carcinoma failed to lead to the production of any increase in the urine indican. In two cases in which the pancreas was involved we failed to observe any indications of sugar secretion, and are not therefore prepared to consider the absence of sugar in the urine as sufficient proof that the pancreas is not involved.*

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*Since the completion of this paper a publication has appeared by Cammidge on the presence of oxidation products of glycerine in the urine and the diagnosis of pancreatic disturbances and of cancer of the pancreas. We are at the present time testing all cases for this reaction, but have not a sufficient amount of material to justify any expression of opinion regarding the value of this reaction for diagnostic purposes.

Destruction of the tissues rich in nuclein, the kidneys, liver, spleen, pancreas, etc., usually leads as might be expected to a slight increase in the proportion of uric acid and phosphates as compared with nitrogen, seldom amounting, however, to an addition of more than .5 per cent to the uric acid nitrogen and .5 to 1 per cent to the phosphates, as compared to the total nitrogen of the urine. Albumin occurs as would be expected in all cases in which the kidneys are involved, and frequently in cases in which the tumor is breaking down, but we have not been able to confirm the observation of von Noorden that it is present in appreciable quantities in over 70 per cent of all cancer cases.

Lactic acid is found occasionally in the urine in cases in which the liver or uterus is involved. We found it continually in one case of cancer of the uterus, but on the other hand it must be stated that in two cases where large metastases were found in the liver at the autopsy no lactic acid had been separated from the urine at any stage of the investigations carried on whilst the patients were under our observation. We have observed the presence of beta-oxybutyric acid and aceton in the urine of patients in a comatose condition, just as is the case in diabetes, and it is interesting to note that in one case at least this phenomenon was associated with a considerable diminution in the apparent alkalinity of the blood. Ehrlich's diazo reaction is, according to Blumenthal, invariably associated with ulcerating tumors. We have obtained a diazo reaction in a very marked degree in two or three cases in which ulceration had set in, but not invariably.

Whilst it would be out of the question to attempt to establish any hard and fast rules whereby a diagnosis could be effected by a study of the urine, considering the limited state of our knowledge at the present moment, a careful consideration of the various factors suggested by Blumenthal and for the most part confirmed by ourselves, has made it possible on one or two occasions to determine the exact location of the tumor. It is, however, well to bear in mind that we have very little evidence of direct activity on the part of the tumor cells leading to any specific effect upon the nature and proportion of the products excreted in the urine, the changes observed being for the most part those which would be induced by some interference with nutrition and the normal processes of oxidation, and also such as would be induced by secondary bacterial infections. A very extensive series of experiments carried out with a variety of group color reagents and also a series of biological tests for specific products which might be definitely associated with tumor cells have so far been resultless in so far as the urine is concerned, but there appears to be no reason why a continuation of work in this direction might not lead to some means of effecting an early diagnosis of cancer.

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ON THE SECRETION OF THE STOMACH IN CANCER AS COMPARED WITH THE NORMAL.

By G. H. A. Clowes, Ph.D. and E. W. Jeffcott, Ph.B.

As a part of the routine work of this Laboratory in the course of the last two years upwards of 200 stomach contents have been submitted to a detailed chemical analysis. Whilst our primary object has been the early recognition of gastric cancer, the materials at our disposal, including as they do some 30 pathologically diagnosed cancer cases, may be utilized to throw some light on the problems of impaired nutrition and disturbed chemical equilibrium associated with this disease.

In a previous paper, an abstract of which has been included at the end of this report, the application of volumetric methods to the analysis of peptic digests has been discussed in detail (see p. 161). The use of various indicators for the direct estimation of acids and akalies will not therefore be further referred to in this paper, except in so far as the normal deviates from the pathological, and also to make comparisons between the rapid volumetric methods usually employed in clinical laboratories, and the accurate but more tedious gravimetric methods which are only available in a well-equipped chemical laboratory.

An attempt has been made to select the most accurate analytical methods and by their application to a series of normal and pathological cases to throw light on the following problems:

1. With given test meals to determine the relationship, if any, existing between the amount of hydrochloric acid secreted and the total of nitrogenous products brought into solution through the action of pepsin on readily precipitated proteids.

2. On the fractionation of the above products of proteid hydrolysis by means of a series of precipitants and the relationship, if any,

existing between such fractions and the titration results. Also the investigation of the value of the phosphotungstic and tryptophan tests for diagnosis of cancer.

3. Regarding the direct secretion of sodium chloride, phosphates, etc., as well as hydrochloric acid, and the possible conversion of hydrochloric acid into sodium chloride through interaction with the sodium salts of organic acids.

4. On the osmotic pressure of the stomach contents at various stages of digestion; the possible role played by physico-chemical phenomena in the mechanism of secretion, and the extent to which toxins or other poisons acting on the cell protoplasm could interfere with the normal state of chemical equilibrium, resulting in an entire or partial suspension of the acid secreting function, or the reverse.

Analytical methods employed.—It seems advisable at this stage to give a brief outline of the analytical methods we have employed so that no confusion may arise:

Volumetric titrations whenever possible on unfiltered as well as filtered contents (see p. 122) were made as follows:

Phloroglucinvanillin drops to faint pink end point on hot porcelain plate.

Alizarin to first color change, to faint red; occasionally when two figures are given, to purple.

Phenolphthalein to first pale pink.

Dimethyl-amido-azo-benzol, lakmoid and Porrier's blue were also employed occasionally.

For details reference must be made to abstract. (See p. 161.)

Estimation of phosphates.—The method at present employed in estimating acid phosphates was also found to be extremely faulty. In order to obtain an estimate of the actual amount of phosphates present and of the effect which they produce upon the titration, use was made of the two following points: (1) that the salt NaH_2PO_4 is converted into Na_2HPO_4 between the alizarin and phenolphthalein end point; and (2) that the salt Na_2HPO_4 may be converted into Na_3PO_4 or its equivalent $Ba_3(PO_4)_2$ between the phenolphthalein end point when that indicator is used alone and in the presence of barium chloride and excess alkali. And since it was shown that two of the three hydrogen atoms of the phosphoric acid are neutralized previous to the phenolphthalein end point in titration and one atom previous to the alizarin end point, allowance may be made for the phosphates by deducting the mean of the result obtained above from the alizarin end point and twice that quantity from the phenolphthalein end point.

The working method employed in all cases to which reference is made in this article is as follows: Filtered stomach contents (25 c.c. if possible) evaporated with a few drops of weak alkali, ashed, extracted with weak acid, filtered, boiled to drive off CO_2, made alkaline and boiled with pure n/10 NaOH to drive off ammonia, made up to definite volume and titrated from phenolphthalein to alizarin and from phenolphthalein to the same indicator after boiling with an excess of alkali in the presence of barium chloride.*

It is needless to say that the assumption of Töpfer that the period of titration from dimethyl-amido-azo-benzol to alizarin is attributable principally to acid phosphates, is incorrect.†

Estimation of chlorides.—In order to obtain accurate data regarding the excretion of hydrochloric acid and chlorides, it was found necessary to evaporate and incinerate the filtered and unfiltered stomach contents in all cases; and also for comparison's sake, to follow the same proceeding with a test meal, the duplicate of that which had been given to the patient. The following estimations were made in all cases in which there was sufficient material at our disposal; a slight modification of the methods of Martius and Lüttke being employed:

a. Chlorine present in the form of neutral inorganic salts other than ammonia by evaporating 10 c.c. of stomach contents and ashing without the addition of alkali, extracting with boiling water and estimating the chlorine, either gravimetrically, as silver chloride in a previously weighed Gooch crucible packed with asbestos, washing with nitric acid, water, alcohol and ether respectively and drying at 100° ; or, volumetrically, by adding to the aqueous extract of ash an excess of n/10 solution of silver nitrate and titrating the unused silver by means of a standard solution of potassium sulpho cyanid, using ferric sulphate as indicator.

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*Am. J. of Pharmacy, vol. 75, Feby. 1903.

†It must be said that the presence of any considerable quantities of phosphates in a test meal is likely to materially affect the titrations, analyses, etc., and is therefore to be avoided whenever possible.

b. The estimation of total chlorine present in the form of inorganic salts of the alkalies, as ammonium chloride and as free or combined hydrochloric acid by addition of excess of dilute sodium hydrate, evaporation, ignition in a platinum crucible as in *a,* subsequent estimation of the chlorine as described above. An alternative gravimetric method which yielded almost identical results consisted in treating a definite quantity of the filtered stomach contents with fuming nitric acid in the presence of an excess of silver nitrate for some days until organic matter was destroyed, diluting with water and filtering through a Gooch crucible as described above. This method gives results almost equal to those obtained in a Carius tube and slightly higher than those obtained by the method previously described, possibly due to the fact that the evaporation and ignition of a carbonaceous residue in an open platinum vessel always leads to a slight loss of chlorides, even though the greatest precautions may be taken not to carry the process of ashing too far. Or a slight difference may be attributable to the inclusion of a very small quantity of organically combined chlorine which would be lost in all probability in the first process but absorbed by the silver nitrate in the second. In any case the loss is so slight that it may be ignored in the physiological chemical work in which the experimental error is necessarily higher than in that of a purely chemical nature.

c. In order to obtain an estimate of the influence attributable to ammonium, a body which seldom occurs in fresh stomach contents in any considerable quantity, an additional portion of 10 c.c. of the original stomach contents was neutralized exactly with n/10 alkali to the phenolphthalein end point, evaporated, ashed and treated as above.

It will thus be seen that by the above process of ashing stomach contents, extracting chlorides and estimating them either volumetrically or gravimetrically, we have obtained from *a,* chlorine present as inorganic salts (not including ammonia); from *c*, chlorine present as inorganic salts + chlorine present as hydrochloric acid free and combined; from *b*, chlorine present as inorganic salts, as hydrochloric acid free and combined and in the form of ammonium chloride. By subtracting *a* from *c* we have the total hydrochloric free and combined with proteids. By adding *a* to *b*—*c* we have the total chlorine present in the form of salts of the strong alkalies and ammonia.

d. An effort was also made to estimate the chlorine present as free hydrochloric acid by treating with barium carbonate, filtering and washing the residue and estimating the amount of barium which had passed into solution, the equivalent being assumed to be a measure of the actual free hydrochloric acid. Some of the results obtained by this method have been recorded but it is inaccurate, and since for reasons which will be referred to later it appears probable that the passage from absolutely free to entirely combined hydrochloric acid is not sharp but gradual and is a question entirely dependent upon the equilibrium of the chemical system concerned, we have not thought it worth while to continue this series of experiments.

Nitrogen.—Nitrogen was estimated in all cases in the filtered stomach contents, the same solution being employed as in the experiments enumerated above.

a. Duplicate quantities of 5 or 10 c.c. were employed for the estimation of total nitrogen by the Kjeldahl process, potassium sulphate being used to raise the temperature.

b. Ten c.c. rendered slightly acid to phloroglucinvanillin drops, if not already so, boiled and filtered and nitrogen estimated in filtrate.

c. Ten c.c. saturated with excess of zinc sulphate crystals, filtered and nitrogen estimated in filtrate.

d. Ten c.c. precipitated with tannin tartaric acid solution, recommended by Effront, filtered and nitrogen estimated in filtrate.

e. Ten to 20 c.c. of the filtered stomach contents were warmed, treated with an equal quantity of hot but not boiling solution containing 20 per cent phosphotungstic acid and 2 per cent sulphuric acid, shaken, allowed to settle and filtered, precipitate being washed with a 10 per cent phosphotungstic acid, nitrogen determined directly in the filtrate by means of the Kjeldahl method.

Estimation of lactic acid.—A qualitative test for lactic acid was first performed by evaporating 10 c.c. of the stomach contents with a small quantity of hydrochloric acid to half its bulk. The solution was then extracted in a separating funnel with 10 c.c. of pure ether. The etherial extract after separating was allowed to flow on to the top of a 1 per cent aqueous phenol solution to which a drop of ferric chloride had been added. At the points of contact of the two fluids a yellow rim was formed in the presence of lactic acid, affording a strong contrast to the purple of the neighboring liquid. A complete transformation into yellow would follow on shaking the mixture provided any appreciable quantities of lactic acid had originally been present. In case the presence of lactic acid was established by this preliminary test, the exact quantity was estimated directly by means of the usual iodine and thio method.

Filtration of stomach contents.—The question as to whether the best results may be obtained with or without filtration of stomach contents is one which is frequently raised in the literature. When an Ewald test meal is employed with a sufficient quantity of water (250 c.c.) no very considerable difference is made in the titration and chloride results, and since it is impossible to estimate the nitrogenous constituents without filtration, it seems advisable to use filtered rather than the original stomach contents, especially in view of the fact that in the clear solution the end points of indicators are rather sharper. On the other hand, we have frequently encountered cases in which for some reason or other an Ewald breakfast was unsatisfactory, as, for example, when a gastrostomy had been performed and the patient was fed through a tube. When a soup meal consisting principally of vegetable proteids is employed, a very material difference is to be observed between the filtered and unfiltered stomach contents. Whilst the osmotic pressure as estimated by the freezing point remains practically constant, not varying by more than .02° C. before and after filtration and the total quantity of chlorine present in the form of acid + salts, estimated by gravimetric methods, is also fairly constant, the titration results show a great difference as do the figures indicating the proportion of salts to hydrochloric acid. In other words, as will be seen by an inspec-

ion of Table XII, a much larger proportion of sodium chloride
asses through the filter than remains behind, whilst a large propor-
ion of the hydrochloric acid is retained by the insoluble or partially
nsoluble proteids left on the filter. In one case, for example, whilst
he total chlorine was 7.3 before and 7.5 after filtering, the chlorine
resent as salts was 2.1 before and 4.0 after filtration, giving differ-
nces of 5.2 and 3.5 for the available hydrochloric acid in the solu-
ion. These figures correspond with the phenolphthalein end points,
hich show 7.0 before and 5.1 after filtration, the difference being
ttributable in both cases to the influence of phosphates and organic
cids, the freezing point meanwhile remaining practically constant,
nly changing from .39° to .40° C. Now, since such differences
ccur very frequently and reach even larger proportions, it must be
orne in mind that filtered stomach contents never actually permit

TABLE XII.

	F P	Phenolpht. end point	Total Cl	Salts Cl	HCl by Diff.	Phosphates
ase						
I Before filtration.	—.39°	7.0	7.3	2.1	5.2	1.6
After filtration..	—.40°	5.1	7.5	4.0	3.5	1.4
II Before filtration.	—.38°	7.5	6.9	1.8	5.1	1.5
After filtration..	—.39°	5.9	7.3	3.0	4.3	1.4

f an estimate of the total hydrochloric acid available for digestive
urposes, and that when regular test meals containing large quan-
ities of animal or vegetable proteids are administered, the error
troduced by filtration may reach such considerable proportions as
entirely mask the presence of quite appreciable quantities of
ydrochloric acid. More especially is this the case if, owing to weak
epsin, the proteids have not been reduced to a point at which they
ass fairly readily through the meshes of an ordinary coarse filter
aper. In one case the unfiltered stomach contents showed both
olumetrically and gravimetrically 2.8 c.c. of hydrochloric acid free
nd combined with proteids, a quantity which, whilst below the nor-
al, was very considerably in excess of that found in the filtrate,
hich was only 1 c.c. We have therefore made a practice of deter-
ining chlorides, phosphates, titrations, etc., in unfiltered contents,

and also in filtered when material permitted. Total nitrogen and its various fractions could only be determined in filtered contents and consequently all figures used in comparison with nitrogen were estimated in the same solution.

1. *Relationship between hydrochloric acid secretion and peptic activity.*—A series of preliminary experiments with artificial peptic digests using varying quantities of hydrochloric acid has shown us that after allowing for soluble nitrogenous products brought into solution by the acid alone the degree of peptic activity is fairly proportionate to the nitrogen present either in the clear centrifuged solution or in the filtrate from which the insoluble residues have been removed, provided a sufficient dilution is maintained and all the working conditions are constant and the period of activity limited to three or at most four hours. We have therefore felt justified in considering the total nitrogen present in filtered stomach contents (after allowing for the soluble nitrogenous bodies present in the original test meal) as proportional to the peptic activity. The determination of this total nitrogen as against the available free and combined hydrochloric acid in some 200 normal and pathological cases (showing equivalents from 1 : 1 to 10 : 1, the large majority lying between 2 : 1 and 3 : 1) has convinced us of the correctness of the view held by Pawlow that no constant relationship obtains between the peptic activity and the hydrochloric acid secretion. The addition of further quantities of hydrochloric acid to pathological contents which showed an absence of free acid frequently led to a greatly increased peptic activity as estimated by the nitrogen brought into solution; a further proof that peptic activity and hydrochloric secretion do not bear any direct relationship to one another.

A method suggested by Benedict for estimating the degree of peptic activity by precipitating the filtered stomach contents with an excess of phosphotungstic acid and centrifuging has not been found to give satisfactory results on comparison with the nitrogen content of the same filtrates.

2. *Fractionation of products of proteid hydrolysis; phosphotungstic and trytophan tests.*—The digestion of proteids in a normal stomach by means of pepsin hydrochloric was supposed until recently to stop

hort at the formation of albumoses and peptones. Boas, Schaeffer, Iammerston, Gamgee, Neumeister, Foster, Beilstein and Hughes ail to make any reference in their works to the formation of bodies epresenting further hydrolysis of the peptones until the latter subtances are submitted to the influence of tryptic digestion. Effront ecognizes the presence of proteids not precipitated by phosphoungstic acid. Zunz refers to substances which fail to give the iuret reaction with copper; also in a subsequent paper to nitroenous bodies giving a biuret reaction which are precipitated by hosphotungstic and not by picric acid. Pfaundler in dealing with ie end products of peptic activity concludes that after precipitating lbumoses with zinc sulphate and peptones with phosphotungstic cid, a residue of nitrogenous products remains in the filtrate which ails to give the biuret reaction which, on hydrolysis with strong cids, may be split up into amido bases and amido acids, the bases ius formed being readily precipitated by means of phosphotungstic cid.

Lawrow and Langstein established the existence of crystalline end roducts of peptic digestion. It must, however, be borne in mind iat such bodies were only obtained by them in vitro after long eriods of incubation.

Reach in a paper on the digestive processes in the stomach, eferring to the work of Zunz, who found 90 per cent of the non-oagulable nitrogenous products present in the stomach contents of ogs in the form of albumoses, started an investigation as to hether the limit of further digestion were 10 per cent or whether ie residual bodies formed were immediately absorbed owing to their wer molecular weight. He carried on his experiments with dogs, nd fractionating the contents after four hours he found from 30 to 6 per cent of albumoses, from 35 to 20 per cent of peptones and rom 31 to 32 per cent of end products, according to the quantity of roteid in the original test meal. The albumoses were precipitated y saturating with zinc sulphate and the peptones by the addition f picric acid to the filtrate from the albumoses.

Emerson, working with Müller, investigated the products obtained y fractional precipitation of the stomach contents of normal individuals and arrived at the conclusion that saturation with zinc sulphate gave a precipitate of albumoses containing 51.5 per cent of the total nitrogen; that further precipitation with warm concentrated phosphotungstic acid gave an additional precipitate of peptones containing 31.4 per cent of the total nitrogen, and that the residual filtrate, amounting to about 17 per cent, representing a more advanced stage of proteolysis, was fairly constant. In the stomach contents of a cancer case he found figures differing very considerably from the above, the albumose fraction being 25 per cent, peptone 47 per cent and the phosphotungstic filtrate over 27 per cent. He claimed that not only were such results to be obtained from the actual stomach contents but that the cancer itself must contain an enzyme capable of carrying peptic digestion to a more advanced stage. Equal weights of cancer tissue, one without heating and the other heated to 80° C. were added to two fibrin hydrochloric acid pepsin digestion mixtures, with the result that after several days the albumose precipitate in the heated test was much larger and the phosphotungstic filtrate much smaller than in that which had not been heated. Toluol was made use of to retard the development of bacteria, but toluol is a comparatively weak disinfectant for a long digestion and apparently no filtration experiments through bougies were carried out, and even had this been the case, previous bacterial development in the tumor might have influenced the result.

We have wherever possible made use of Ewald breakfasts or plain proteid test meals in stomachs which had previously been washed out with distilled water. After removing and filtering the contents when the quantity was sufficient (in addition to the titrations and the determinations of chlorides, phosphates, etc., referred to in another section) the nitrogen precipitate by boiling was determined, the nitrogen precipitate on saturation with zinc sulphate, the nitrogenous bodies precipitated with phosphotungstic and those present in the phosphotungstic filtrate. In certain cases the precipitate with bromine and the precipitate by means of tannin-tartaric acid solution were also determined. The precipitate on boiling was usually from 3 to 5 per cent. That obtained by saturating with zinc sulphate under normal conditions from 40 to 55 per cent, a result which was frequently fairly in agreement with that obtained from the tannin-tartaric acid. The phosphotungstic acid precipitate was usually about 80 per cent, leaving the phosphotungstic acid filtrate containing between 17 and 20 per cent of the total nitrogen.

In the accompanying Table XIII the results of fractionating normal stomach contents according to the above scheme are recorded. Three cases are typical of many others and will suffice as an illustration of normal results. Column 1 gives in cubic centimeters of a $\frac{1}{10}$ N solution the total nitrogen in 100 c.c. of stomach contents. Column 2 the nitrogen content of the bodies which have been precipitated by boiling. Column 3 the nitrogen of the zinc sulphate precipitate obtained by saturating the solution with that salt and subsequently washing the precipitate with a saturated solution. Column 4 gives the nitrogen of the phosphotungstic acid precipitate obtained by treating 10 c.c. of the warm stomach contents with 10 c.c. of the 20 per cent phosphotungstic acid solution (containing 2 per cent of sulphuric acid) referred to above. Column 5 gives the results of a similar precipitation with phosphomolybdic acid and 6 the nitrogen content separately determined of the filtrate from the phosphotungstic acid precipitate. It will be seen that in the first two cases the portion precipitated by boiling is about 3½ per cent; by saturating with zinc sulphate over 50 per cent; by precipitating with phosphotungstic or phosphomolybdic acid over 80 per cent, the resulting filtrate being about 19 per cent. In the third case, whilst the precipitate on boiling with phosphotungstic acid was about the same as in the first case, the zinc sulphate filtrate was considerably smaller, being about 37 per cent. The titrations of the same materials in Case I showed for 10 c.c. 3.5 c.c. to phloroglucinvanillin drops end point;

TABLE XIII.

Normal case	1	2	3	4	5	6
I.........	147	5	79	119	120	27
II.........	160	6	85	129	127	30
II..........	144	5	53	117	118	26

4.4 c.c. to the alizarin end point and 7.1 c.c. to the phenolphthalei end point, the difference of 2.7 between the two latter correspondin very closely with the phosphotungstic filtrate, a point which wil be referred to at a later stage. The end point 7.1 c.c. for phenolphthalein after deducting .6 c.c. attributable to phosphates corresponds fairly with 6.3 c.c. of available hydrochloric acid estimate gravimetrically. The hydrochloric acid present is consequentl equivalent to rather less than a half of the total nitrogen, a relationship which very frequently obtains.

In Table XIV a series of the most widely deviating pathologica cases has been selected and will suffice to prove that not only is th above relationship of normal cases by no means constant, but als that certain pathological cases do not necessarily deviate from th normal as might be supposed from the literature. Cases 1 to 5 were microscopically and clinically and subsequently by autopsy diagnosed as cancer of the stomach, and it will be seen at a glance that while three show a high percentage of phosphotungstic acid filtrate the remaining two are perfectly normal, being under 20 per cent. On the other hand, cases 6, 7 and 8, which are not cancerous, 6 and 7 being gastric ulcers and 8 a case of hyperchlorhydria, may be selected as illustrating an extreme deviation from the normal, phosphotungstic filtrates being 46, 24 and 65 per cent respectively. The appended figures showing the amount of hydrochloric acid physiologically available in the stomach are simply introduced to show that abnormalities may occur either in the complete absence or in the presence of an excess of free hydrochloric acid.

TABLE XIV.

	1	2	3	4	5	6	7	8
Total nitrogen...........	158	185	162	110	150	116	210	91
Nitrogen in zinc sulphate ppt....................	70	86	98	30	45	55	35	10
Nitrogen in phosphotungstic filtrate.............	25	47	17	36	37	53	47	60
Physiologically available hydrochloric acid......	18	23	Nil	12	Nil	66	45	90

After determining the nitrogen of the phosphotungstic filtrate as against the total nitrogen in some 200 stomach contents after proteid or Ewald test meals, we are by no means inclined to agree with Emerson in assuming that a greatly increased phosphotungstic filtrate may be looked upon as indicative of cancer of the stomach. The largest recorded analyses of 60 and 70 per cent referred to above were obtained in cases of hyperacidity. On the other hand, igures in excess of the normal were obtained in certain cases in vhich hydrochloric was practically absent and the bacterial enzymes had evidently played an active part. Whilst normal stomach con-ents, generally speaking, show from 18 to 22 per cent after from wo to three hours digestion, and cancer cases frequently show as much as 40 per cent, we have had six cases of cancer of the stomach onfirmed subsequently by autopsies, which showed from 10 to 20 er cent phosphotungstic filtrate, and 2 cases of gastric ulcer have iven results even in excess of the highest obtained in cancer. The ame relationship is found to obtain in dealing with Ewald's ryptophan reaction, which will be referred to later.*

Whilst there does not appear to be any constant relationship etween the available hydrochloric acid present in a gastric digest and the total quantity of nitrogen in the solution (which may be taken as a criterion of the peptic activity) other definite proportions occur frequently in the various nitrogenous fractions which can scarcely be overlooked. When digestion has been allowed to take its proper course in the presence of a normal proportion of pepsin and hydrochloric acid for at least two to two and a half hours, whilst the total range of titration varies within very wide limits, as was shown in a previous paper referred to above, that portion lying between the alizarin and phenolphthalein end points is, after allowing for phosphates, frequently about 18 to 20 per cent of the total nitrogen equivalent. The phosphotungstic filtrate is also, as stated above, frequently about 20 per cent of the total nitrogen; consequently a very close equivalence exists between the range from alizarin to phenolphthalein on titration and the phosphotungstic filtrate. (See Table XV.)

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*In considering the apparent tendency to advanced proteid decomposition cancer as compared with the normal resulting in an increased phospho-ngstic filtrate, it is interesting to note the result of some experiments rried out in this Laboratory in the winter of 1902, having in view the oduction of an anti-pepsin by means of rabbit serum. Von Dungern has tained an anti-pepsin by making use of geese and failed to obtain any action from the ordinary laboratory animals. Our results agree entirely th his in the main, the sole difference being the tendency to an increased osphotungstic filtrate in those cases of peptic digestion to which the immune um had been added, in comparison with those containing normal rabbit um. This would indicate either a stimulus of the secondary digestive ction of the stomach or a neutralization of some normal constituent, the ction of which would be to restrain any tendency to the continuation of tic digestion beyond a certain point. It is conceivable, although scarcely bable, that such a body controlling the digestive processes and protecting cells from the action of peptic and other enzymes may be entirely troyed in certain cancer cases. We are not, however, inclined to the ef that this is actually the case, considering it far more probable that the rved results are due to secondary antolytic or bacterial enzymes.

TABLE XV.

Case	1	2	3	4	5	6	7	8	9	10	Avg.
Alizarin to phenolphthalein	25	19	30	26	43	31	17	31	21	35	....
Phosphates	4	2	5	4	5	4	1	5	3	5	....
Difference	21	17	25	22	38	27	16	26	18	30	24.0
Total nitrogen	113	107	131	120	228	135	80	127	104	144	128.9
Phosphotungstic filtrate	23	19	23	27	40	26	18	24	19	27	24.6

In this table are recorded the titration differences between alizarin and phenolphthalein, the phosphates determined independently which should be deducted from the previous quantity in order to form a fair estimate of the influence attributable to some specific basic group combined with hydrochloric in the solution. The total nitrogen and the nitrogen of the phosphotungstic filtrate from 100 c.c. of stomach contents were also recorded in their n/10 equivalents. It will be seen at a glance that the closest relationship exists between the corrected titration and the phosphotungstic filtrate; further, that both these quantities are slightly less than one-fifth of the tota nitrogen.

This relationship obtains in about 40 per cent of the pathologica cases investigated, but is subject to wide and unaccountable varia tions which preclude its utilization as a means of diagnosis. It has been stated previously that where hydrochloric acid is deficient, or from some other cause stagnation or bacterial decomposition has occurred, the widest range of variation of titrations is encountered, and at the same time the phosphotungstic filtrate is likely to rise considerably above 20 per cent, its normal value. But the titration fraction from alizarin to phenolphthalein is not necessarily affected at the same time as the phosphotungstic filtrate, very frequently one remaining directly proportionate and the other being greatly increased or diminished, and that without any apparent rule, certain cases of well-marked cancer of the stomach exhibiting normal proportions and others deviation in one direction or the other, more generally, however, in the matter of the phosphotungstic filtrate.

Whilst the residual phosphotungstic filtrate shows a certain relaionship with the titration results, it has not been found possible to race out any connection between the so-called albumose fraction, precipitated by zinc sulphate, and the portion between zinc sulphate nd phosphotungstic acid supposed to represent peptones and amido bases and the titration results. The period from drops to alizarin which probably represents some form of weakly combined hydrohloric fails to show, as might be expected, any proportionality with ny of the above mentioned fractions of the nitrogenous constitunts and can not therefore be made use of as a means of differntiating between the normal and pathological, except in those cases n which an abnormal bacterial disintegration of proteids resulting n the liberation of free acid groups shows titration results out of ll comparison with the mineral acids actually present.

The observation made by Winternitz, Malfatti and Glaessner independently that tryptophan, one of the final products of tryptic digeson, is to be found amongst the products of peptic digestion, led o the examination of normal and diseased stomach contents for this ubstance as a possible means of diagnosing pathological conditions. nce tryptophan has been identified by Hopkins and Cole as an indolmino propionic acid and is generally associated with leucin, tyrosin nd similar products of final proteid digestion, and possesses in mmon with the latter the property of not being precipitated from a weak acid solution by means of phosphotungstic acid, its presence or absence might be expected to correspond with the increase or decrease in the size of the phosphotungstic filtrate. Glaessner tried the effect of feeding patients with proteid test meals, removing the contents and testing with bromine water in the presence of small quantities of acetic acid. He experimented with 36 cases, 12 of which were cancer, and obtained positive results in only 4 cases, all of which were highly acid and only one carcinoma. The same author tested the effect of adding stomach contents of cancer cases to a normal peptic digest, obtaining a positive result in only one case. On the other hand, he found that the addition of the actual tumor material to a normal pepsin-hydrochloric digestion led in the course of 24 hours to a strong tryptophan reaction with bromine, other tissues, normal stomach membrane, liver, etc., giving negative results. Erdmann and Winternitz in discussing the proteinochrom (tryptophan) occurring in the stomach contents, recommend the use of chlorine instead of bromine on account of the disturbing effect of an excess of the latter substance. They failed to obtain the reaction in any normal or pathological cases other than cancer, except where stagnation of the contents had led to secondary decomposition. In carcinoma they obtained a positive reaction by the addition of chlorine water, drop by drop, to filtered stomach contents in the large majority of cases, even in the absence of bacterial decomposition.

In the course of the last twelve months we have tested on suspected cancer cases both with chlorine and bromine in weak acid solution. Whilst stagnant stomach contents, especially in the presence of a large excess of acid, will frequently give a faint reaction, as will stomach contents in which bacteria have suffered no check owing to the absence of free hydrochloric, we have never succeeded in obtaining any marked reaction in cases in which the stomach had been carefully washed with distilled water before administering the test meal.

We have further attempted to follow out the method made use of by Emerson for the phosphotungstic filtrate and Glaessner and subsequently Erdmann and Winternitz in studying the trytophan reaction, of adding cancer of the stomach tissue immediately after the operation to artificial peptic digestion mixtures which were allowed to stand over night in the incubator. Even then the phosphotungstic filtrate was not necessarily greatly increased and the trytophan reaction was only obtained after incubation periods varying from 24 to 48 hours in some 40 per cent of the cancer cases. It was further observed that far the strongest reaction with chlorine was obtained when in the absence of disinfectants foreign organisms were allowed to develop whether the tissue employed were of a cancerous origin or not. We have not up to the present examined a sufficient number of cases to be justified in offering a positive opinion on the subject, but it can not be said that the evidence so far obtained would justify the assumption that the phosphotungstic and tryptophan reactions may be looked upon as indicating the presence in cancerous tissue of some peculiar enzyme capable of carrying proteid digestion to an abnormally advanced stage in the presence of free acids. The fact that the best reactions were invariably obtained in those cases in which advanced bacterial decomposition had already taken place inclines us to the belief that any such reaction is merely secondary and is not to be attributed to the direct influence of the carcinomatous cell and its contents. This is the more unfortunate since the existence of such a body capable of decomposing peptones with the formation of some of the final products of tryptic digestion might well explain the disturbances in equilibrium resulting in the suspension of hydrochloric acid secretion, etc., observed in cancer cases.

SECTION III. *Secretion of Chlorides, Hydrochloric Acid, Etc., in Cancer.*

Reissner in dealing with the estimation of hydrochloric acid in the stomach contents points out errors in practically all existing methods. He finally makes use of a modification of that of Martius and Lüttke. He decides after numerous experiments with free hydrochloric acid in the presence of chlorides, phosphates, peptones, organic acids, etc., to determine the physiological available hydrochloric acid by a method similar to that which we employ, the exception being that he titrated filtered stomach contents to the lakmus end point instead of phenolphthalein, and consequently found rather larger proportions of the chlorides present as ammonium salts. The method depends, as does that which we have employed, upon the assumption that in stomach contents, which are evaporated, charred and partially but not completely ashed, a point can be determined empirically at which the chlorides originally present will still be entirely retained and the hydrochloric acid which was present in the free state and combined with proteids or ammonia completely evaporated. Further, that by addition of alkali to a given end point one may include all the acid present in the free state and combined with weak bases present in the proteid molecule, without including that which was present in combination with ammonia; and that an excess of alkali will certainly include all chlorine originally present of whatsoever sort, provided too high temperatures are not employed in ashing. If the temperature is carried too far there can be no question that small quantities of chlorides are lost by direct vaporization; also that acid phosphates interacting with sodium chloride would eventually liberate hydrochloric acid. Reissner apparently attributes the low figures which he obtains partially to insufficient time permitted for the reaction to reach equilibrium after the addition of the exact amount of alkali required to neutralize the hydrochloric acid, or an excess. Also he suggests the possibility of neutral chlorides as calcium chloride interacting with acid potassium phosphate with the liberation of potassium chloride and hydrochloric acid, which is volatile. The actual state of equilibrium eventually established is entirely dependent upon the concentrations and the temperatures employed at various stages of the analysis. The simplest solution of such a problem is to avoid test meals containing any large quantities of phosphates, organic acids, ammonia, etc., and to make exact analyses of such test meals, a procedure which we have systematically adopted.

It has been noted in a previous section of this paper that the secretion of pepsin in the stomach is less readily influenced by the presence of a tumor than is that of free hydrochloric acid. Tests for the presence of free hydrochloric by means of phloroglucinvanillin drops, or of hydrochloric combined with proteids by the gravimetric methods referred to above may show absolutely negative results before the secreting cells have been seriously interfered with by the development of the tumor, a fact which excludes a purely histological explanation of the phenomena of impaired hydrochloric acid secretion. This extreme sensitiveness of the hydrochloric as compared with the pepsin secreting function of the cell is remarkable in view of the fact that mineral salts are chemically far less susceptible to changing conditions than are the enzymes and ferments. On the other hand, salts and mineral acids are electrolytes and consequently play a very important role in the establishment of osmotic equilibrium, and any disturbing influence in this direction may well be expected to affect them more easily than pepsin, a body which is presumably possessed of a large molecule and is incapable of playing any considerable role in osmosis.

Having observed in the course of our gravimetric work on the chlorides of the stomach contents that abnormally large quantities of sodium chloride are frequently found associated with very minute quantities of hydrochloric acid, the first point to be determined was to what extent sodium chloride might be secreted directly in the stomach in the place of free hydrochloric; also to what extent the hydrochloric when secreted might be directly neutralized by contact with the sodium salts of organic acids produced as products of suboxidation in the tumor itself. A long series of experiments was carried out on cancerous and non-cancerous cases as follows: Series of test meals were given containing definite quantities of sodium chloride, the greatest precaution being taken to remove all traces of chlorides from the stomach before commencing the experiments. After the usual period had been allowed to elapse the contents were removed, the residues washed out and the total quantity carefully analyzed. In practically all cases, both normal and abnormal, more sodium chloride was present in the stomach than had actually been introduced with the test meal. In normal cases this proportion seldom exceeded 5 to 10 per cent of the total amount of chlorine present, but in cancerous cases, especially those in which the tumor had already commenced to disintegrate, quantities of sodium chloride far in excess of those actually introduced and sometimes nearly equivalent to the normal proportion of hydrochloric acid were encountered. This was not, however, always the case, a total absence of hydrochloric acid being associated occasionally with an almost total absence of sodium chloride over and above the quantity actually introduced. Any neglect in the removal of the stagnant contents previous to administration of the test meal invariably showed a high proportion of sodium chloride, possibly owing to the increased volume of fluid affording an increased osmotic tension and increased secretion of salts and the presence of the sodium salts of organic acids which would react with any hydrochloric acid secreted. In one case, primary cancer of the oesophagus with involvement of the stomach and liver, a gastrostomy having been performed, the patient was fed entirely through a rubber tube. Such a case afforded an admirable opportunity of determining the effect exerted on the secretion of hydrochloric acid, sodium chloride and certain other mineral salts by introducing varying proportions of those substances directly into the stomach; the point being determined at which, owing to the high osmotic tension of the fluids introduced, no secretion was to be observed. It is not possible to employ an Ewald test meal in such a case, owing to the tendency of the bread, however finely divided, to block up the tube. A soup meal was therefore employed, determinations of nitrogen and chlorides being made in advance. The stomach was washed out with water twice before the administration of each meal. The meal was removed at the end of a period of an hour and a half and all residual material washed out with a fresh quantity of water. By determining the quantity of nitrogen present in the stomach contents it was possible to detect any loss which might have taken place through passage of the contents into the intestinal tract. Organic acids were not present in any large quantity, probably owing to the fact that all residues of meals left in the stomach after four hours were removed.

An inspection of Table XVI demonstrates fairly conclusively that sodium chloride, as such, must have been secreted directly by the cells of the stomach wall, especially in those cases in which no sodium chloride had been included in the diet. On the other hand, a large amount of sodium chloride in a test meal was usually associated with a loss of that substance in the stomach, and since the loss directly into the intestine was extremely small and could be allowed for, it must be supposed that chlorides actually found their way into the system by way of the stomach membrane.* The larger the quantity of hydrochloric introduced the smaller was the actual secre-ion of that substance and the larger the proportion of sodium chloride produced, indicating a possible reversing of the ordinary hydrochloric secretory functions, alkalies being in some way obtained

TABLE XVI.†

DATE	TEST MEAL			STOMACH CONTENTS		
	NaCl	HCl	Total	NaCl	HCl	Total
Oct. 8....	560	Nil	560	210	210	420
9....	500	"	500	225	150	375
10....	450	"	450	240	70	310
12....	315	"	315	137	140	277
13....	90	"	90	140	70	210
29....	420	"	420	237	87	324
31....	500	"	500	250	60	310
ov. 3....	175	175	350	175	150	325
4....	280	200	480	230	170	400
5....	20	Nil	20	75	125	200
6....	10	180	190	90	160	250
7....	10	200	210	80	200	280
8....	35	Nil	35	80	120	200
9....	875	"	875	410	70	480
10....	10	290	300	100	160	260

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* This observation is in agreement with that of Bonninger, who found that e addition of large quantities of sodium chloride interfered with the normal cretion and reduced the action of pepsin on proteids.

† Table XVI gives a comparison of the proportions of sodium chloride and drochloric present in an exact duplicate of the original test meal, and of the lium chloride and hydrochloric acid present in the stomach contents removed er an hour and a half. The materials in both cases were unfiltered. The ures represent cubic centimeters of n/10 silver nitrate solution equivalent he total quantity of chlorine present either in the form of sodium chloride hydrochloric acid, the estimation being carried out as described in the lytical section. The results observed may be converted into grams of orine by multiplying by the factor .00365. The series of experiments was ducted from October 8 to November 10, and at the autopsy of the case on vember 25 it was discovered that almost the entire wall of the stomach was trated with carcinoma.

from the stomach walls and a certain proportion of hydrochloric acid actually penetrating in the reverse direction.

The tendency to establish a state of equilibrium not only as regards the total volume and osmotic tension but also between the sodium chloride and the hydrochloric acid is very remarkable in spite of the fact that the infiltrating carcinoma had invaded a great portion of the secreting surface. This tendency is still to be observed in the later experiments carried out less than a fortnight before the death of the patient. In the early experiments the introduction of a large amount of salt was generally associated with the conversion of a considerable portion into hydrochloric acid, the amount of hydrochloric formed in this way greatly diminishing as the disease progressed. On November 3 and 4, when fairly balanced proportions of the sodium chloride and hydrochloric acid were introduced, the state of equilibrium was not very materially altered. On November 5 and 8 very small quantities of sodium chloride and no hydrochloric being introduced in the test meal, sodium chloride and hydrochloric acid were apparently secreted in the proportions of about 1 : 2. November 6, 7 and 10 practically no sodium chloride and large quantities of hydrochloric in excess of the normal introduced in the test meal resulted in a slight loss of hydrochloric acid and a considerable increase in the amount of salt. The high concentration of sodium chloride in the test meal on November 9 was associated with a considerably reduced concentration of salt in the removed stomach contents, and as on October 29 and 30 the introduction of a very large amount of salt with a high osmotic pressure seemed to result in the passage of chlorides through the stomach wall into the system and a slight formation of hydrochloric acid in the stomach either through secretion or the loss of sodium ions, the quantity of acid formed being considerably less than under normal circumstances when moderate or even extremely small quantities of chlorides were administered, as on November 5 and 8.

Through the whole series the tendency of this particular stomach to establish an equilibrium at about 250 units, or .9 to 1 gram of chlorine, is evident. The state of the patient rendered the completion of this series of experiments impossible, and the experimental errors, although reduced to a minimum, introduced sufficient variations to preclude the possibility of working out any mathematical relationships.*

If the test meal was permitted to remain a sufficiently long time in the stomach an osmotic equilibrium representing from .4 to .45° C. was generally established through the interchange of water and salts between the stomach contents and the system. An arrest of the process at the end of an hour to an hour and a half showed, both in cases of high and low osmotic tension of the original test meal, a tendency to the establishment of an equilibrium as near the average as possible, a finding which is more or less in accord with that of Pfeiffer but scarcely in agreement with that of Strauss, who considers that the secretory functions of the cells of the stomach membrane far exceed their tendency to absorb.

It would not of course be possible to base any final conclusions on a single case of this nature, but the observation that a diminished secretion of hydrochloric acid is frequently associated with an increased secretion of sodium chloride justifies the assumption that the failure of the cells to secrete a normal proportion of hydrochloric acid is only in part attributable to a diminished secretory function and may to a certain extent be accounted for as due to faulty or incomplete dissociation of sodium chloride in the blood and the cells concerned, probably due to the influence of sub-oxidation products and toxins produced in, and excreted from the tumor cells. This view tends to support the observation regarding the abnormal decomposition of proteids in cancer stomachs referred to in a previous section. It is also in agreement with the observed diminution in alkalinity and increase in osmotic pressure and sodium chloride content of the blood referred to elsewhere in this report, phenomena which are in all probability directly dependent upon some interference with metabolism resulting in incomplete and faulty oxidation of food stuffs.

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* Similar series of experiments covering shorter periods were carried out on other cases, the same tendency to establish a definite state of equilibrium between NaCl and HCl being observed. In certain cancer cases sodium chloride can apparently almost replace hydrochloric acid in molecular equivalence.

Leo Schwarz, in a valuable paper dealing with the question of chlorine starvation and the alkalinity of the urine, shows that a marked increase of the latter quantity is brought about by introducing sodium chloride and sodium bromide into the stomachs of dogs, bromine apparently functioning as an equivalent for chlorine, whilst nitrates and other mineral salts produce no marked effect on the state of equilibrium of the system.

Reissner concludes that a fairly uniform chlorine content of the stomach is generally to be observed, and that the absence or presence of free hydrochloric and hydrochloric combined with proteids is more or less dependent on the extent to which the original hydrochloric secreted had been neutralized with sodium. He evidently considers that the hydrochloric acid is secreted in any case and subsequently a portion of it neutralized. Our experiments would rather lead us to suppose that a certain proportion, in some cases a considerable amount, of sodium chloride was actually excreted as well as hydrochloric acid; and whilst we have frequently found abnormally large proportions of sodium chloride in cancer cases, as was stated above, other cases of cancer have also been recorded in which practically no hydrochloric acid or sodium chloride was to be found. Whilst in normal stomach contents the phenolphthalein end point, after allowing for phosphates, gives a fairly accurate estimate of the total available hydrochloric acid, as will be seen from Table XVII, this is in no sense true in pathological cases. As was stated in an article on indicators published recently and abstracted in this report, the discrepancies observed can scarcely be accounted for as due entirely to the presence of fatty organic acids and must partially be attributed to the abnormal decomposition of the proteids themselves.

It will be seen that in the first part of this table the available physiological hydrochloric acid estimated by gravimetric methods when added to the acid phosphates is about equal to the phenolphthalein end point, but this is in no sense the case in the cancer results recorded in the next section, although certain of them show very little discrepancy, whilst the big differences observed in the first five cases are in each case partially to be attributed to the influence of lactic acid. There is a residual difference which must be attributed to some type of proteid decomposition. In the last section giving non-cancerous pathological cases it will be seen that certain discrepancies are to be observed, which in view of the fair amount of hydrochloric acid present, can scarcely be attributed to bacterial decomposition.

TABLE XVII.

Non-Cancer Cases.

CASE	1	2	3	4	5	6	7	8	9	10	11	12
Free and combined HCl	7.7	11.1	9.0	9.4	8.0	5.6	7.1	3.6	2.6	4.1	6.6	6.4
Total phosphates	Nil	Nil	1.5	.6	.3	.4	.8	.8	.4	.4	.2	.6
Sum	7.7	11.1	10.5	10.0	8.3	6.0	7.9	4.4	3.0	4.5	6.8	7.0
Phenolphthalein end point	7.6	10.9	10.6	9.6	8.0	5.9	8.3	4.5	2.8	4.6	6.6	7.1
Difference	—.1	—.2	+.1	—.4	—.3	—.1	.4	+.1	—.2	+.1	—.2	+.1

Cancer Cases.

CASE	1	2	3	4	5	6	7	8
Free and combined HCl	.0	.4	.8	.0	4.0	4.8	2.7	6.2
Total phosphates	.8	.6	.5	.2	1.0	.5	.4	.8
Sum	.8	1.0	1.3	.2	5.0	5.3	3.1	7.0
Phenolphthalein end point	8.8	6.8	4.9	1.1	10.0	5.4	3.3	8.4
Difference	+8.0	+5.8	+3.6	+.9	+5.0	+.1	+.2	+1.4

Pathological Cases Other than Cancer.

CASE	1	2	3	4	5	6	7	8
Free and combined HCl	14.4	8.0	11.0	6.8	7.3	6.1	3.6	2.4
Total phosphates	1.5	.8	1.1	.9	.6	.5	1.0	.4
Sum	15.9	8.8	12.1	7.7	7.9	6.6	4.6	2.8
Phenolphthalein end point	17.0	10.0	15.2	10.1	9.6	4.0	9.0	5.8
Difference	+1.1	1.2	+3.1	+2.4	1.7	—2.6	+4.4	+3.0

IV. *On the osmotic pressure of stomach contents.*—A series of freezing points made on 75 normal and pathological stomach contents gives results varying from .15° to .8° C. lowering of the freezing point. No direct relationship obtains between the freezing point of the stomach contents and the specific gravity, as is the case in urine, owing presumably to the large quantity of starch, etc., usually administered in a test meal. There is not the same difference to observe between the osmotic pressures of the contents of normal and cancer stomachs as is found in the titration values, presumably owing to the increased proportion of salts, sodium chloride and phosphates which are capable of replacing the free acid in maintaining the osmotic pressure in cases in which cancer has already made considerable advance.

The appended Table XVIII gives the comparison of the osmotic pressure of test meals and stomach contents after one and one-half hours, carried out on the same case as that referred to in previous Table XVI. The tendency to establish an equilibrium of osmotic pressure represented by a lowering of the freezing point of about .4° may be observed, all cases in which the osmotic pressure was higher that figure being diminished and when lower increased.*

Table XVIII.

Lowering of Freezing Point in Degrees C., Indicating Osmotic Pressure.

Osmotic pressure of meal . . .	1.27°	1.20	.93	.91	.67	.51	.36	.29	.27	.19
Osmotic pressure of contents after one hour and a half. . .	.87°	.79	.72	.67	.58	.47	.41	.39	.39	.34

Summary and Conclusions.

1. A series of exact gravimetric determinations makes it apparent that developing cancer of the stomach is generally associated with diminishing secretion of hydrochloric acid in that organ, a point being very frequently reached at which no free mineral acid is to be found.

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*A series of determinations of freezing point and conductivity in comparison with specific gravity, titrations and the amounts of certain chemical constituents of the stomach contents, blood and urine of a series of pathological cases are at present being carried out in this Laboratory and will be reported upon as soon as the material at our disposal is sufficient for that purpose.

2. This decrease in the proportion of hydrochloric acid is frequently associated with an increase in that of the neutral chlorides, and to a certain extent of the phosphates, the evidence inclining rather to support the assumption that the neutral salts are themselves secreted directly from the cells of the stomach wall, and not, as has been assumed by some authors, being produced as the result of interaction between hydrochloric acid normally secreted and the sodium salts of organic acids removed from the disintegrating tumor.

3. When proper precautions are observed in washing the stomach in advance, administering a normal test meal and removing the entire meal after a reasonable period of time has elapsed, no appreciable quantities of lactic acid are to be found except in those cases in which advanced secondary decomposition of the tumor mass has already set in.

4. The secretion of pepsin in normal proportion is maintained to a later stage than is that of hydrochloric acid, a fact which may frequently be demonstrated by the addition of acid to materials derived from a cancerous stomach, when a marked increase in digestion may be noticed on incubation.

5. The proportion of nitrogen present in filtered stomach contents which bears a fairly close relationship to the existing digestive activity is not in any sense proportional to the chlorine secretion.

6. The so-called phosphotungstic filtrate is usually about 20 per cent of the total nitrogen in well-digested mixtures, but may rise as high as 50 and 60 per cent in cases in which, owing to the presence of abnormally large quantities of hydrochloric or the entire absence of that substance, digestion has taken an abnormal course, and is by no means confined exclusively to cancer cases.

7. The titration of stomach contents by means of phloroglucin-vanillin drops, alizarin and phenolphthalein with a separate estimation of acid phosphates, permits in a majority of cases, of a fair estimate of the free hydrochloric to the phloroglucinvanillin drops end point and of the total available hydrochloric acid to the phenolphthalein end point; and the range from drops to alizarin and alizarin to phenolphthalein usually bear a certain relationship to nitrogenous products present in solution.

8. The period from alizarin to phenolphthalein after correcting for phosphates is normally about one-fifth of the total nitrogen, but shows wide variation in certain pathological cases.

9. The influence of the phospates either present in the test meal or secreted by the cells of the stomach may be estimated by a process referred to in a previous publication. (See page 119 of this report.)

10. The tryptophan reaction supposed to indicate cancer of the stomach has not been found to give uniform or conclusive results.

In conclusion it must be said that no individual characteristics in the chemical analysis of stomach contents can be looked upon as having any absolute diagnostic value. Only the intelligent combination of the various analyses with the microscopical examination taken in conjunction with the clinical side of the case can be looked upon as in any sense conclusive. On the other hand, something may be gained by the application of work of this nature to the solution of problems associated with the secretion of acids, etc., in the stomach and the suspension of this function under pathological conditions. It is excessively difficult to prove the existence of any specific toxic bodies in tumors, although from work on blood corpuscles recently carried out it appears probable that such conditions actually exist. In any case the great proportion of the toxic effects observed in cancer cachexia may be attributed to the influence of sub-oxidized products produced under the abnormal conditions of metabolism of the cell. The failure to secrete hydrochloric acid might well be due to some slight interference with the equilibrium of the chlorides, phosphates, carbonates, etc., in the blood serum. A slight increase in the viscosity of the cell membranes, increased difficulty in diffusion of chlorine ions in one direction and carbonate ions and others resulting from the oxidation processes in the opposite direction, might well result in such a disturbance of equilibrium as to lead to the suspension of such a sensitive function as the hydrochloric acid secretion. The observed increase of chlorides in the blood, decrease of alkalinity and increase in osmotic pressure, etc., associated with decreased secretion of certain salts in the urine all point in the same direction, but even when it is established that such conditions actually exist in cancer, it is extremely difficult to differentiate between cause and effect. It is for clinicians, aided by pathologists, and to a certain extent by chemists, to differentiate sharply between that which is cause and that which is effect in phenomena of this nature. Either by interference with the physico-chemical equilibrium of the cell a stimulus to proliferation might be induced or the agent producing the stimulation may at the same time play an important role in the production of the phenomenon referred to above, which might consequently be looked upon as a mere secondary reaction. This is a point upon which it is not possible to throw any considerable light at present.

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ON THE REDUCTION OF STARCH IODIDE SOLUTIONS BY MEANS OF THE AQUEOUS EXTRACTS OF TISSUES.

By G. H. A. Clowes, Ph. D.

In a recent publication on chemical methods of differentiating hypernephromata from other tumors of the kidney, the statement is made by Croftan that the reduction of a starch iodide solution may be looked upon as characteristic of the extracts of the adrenals and also of hypernephromata, tumors supposedly derived from adrenal tissue. We are not able to confirm this observation of Croftan's. And further, bearing in mind the known decolorizing effect of ammonia and all alkaline solutions, lecithin and unsaturated fatty acids and even the proteids themselves on iodine solutions, it seemed extremely improbable that such a method as that suggested could have any differential value. It appeared to us necessary to submit the method suggested to a series of severe analytical tests before accepting it as a standard.

Croftan makes no exact statements regarding the strength of the iodine solution which he employed. He merely points out that too great a concentration should be avoided in order that a reaction may be obtained. We therefore carried out a series of experiments in which a n/500 solution of iodine and an equally strong solution of sodium-thio-sulphate were employed as standard solutions and a one per cent starch solution as indicator.

The blood serum, blood corpuscles, urine and various organs of a series of animals, as well as post-mortem material from human beings, were tested quantitatively regarding their reducing power. Our procedure was as follows: a given weight of certain tissue was ground in a mortar with a small quantity of sand and distilled water, repeated extracts being made in this manner until the total volume of fluid after filtration was equal to 100c.c. One c.c. of the starch solution was then added to each of three twenty-five c.c. portions; one was titrated directly by means of the dilute iodine solution; a second was first boiled and then titrated; to a third five c.c. of a n/50 sulphuric acid solution were added before titration. In dealing with the majority of extracts it was observed that no very sharp end point was obtainable, but comparable end points might be found in all cases; that is to say, the first end point was that point at which the iodine was no longer completely decolorized, leaving a faint pinkish-blue tinge through the solution for the space of a few seconds. On titrating further an end point was obtained where a distinct blue remained constant for a space of fully a minute. The solution was usually still capable of effecting a further decolorization of two or three c.c. of iodine solution, provided sufficient time was allowed to elapse, but we found that it was most satisfactory for general purposes to employ the second end point indicated above, at which the blue remained constant for about a minute.

The first materials treated in this manner were derived from a case of cancer of the stomach, in which the patient died as the result of a rupture of the stomach wall and in which it was possible to perform an autopsy within three hours after death. The following table represents the reducing power of the extracts of a series of organs, estimated in cubic centimeters of a n/500 iodine solution for each gram of tissue extracted. The first column represents results obtained on direct titration with iodine in the presence of starch in the cold; the second after treatment with a sufficient amount of dilute acid to render the solution acid to all indicators, and the third after boiling sufficiently to coagulate proteids.

It is interesting to note that using material from different portions of the tumor, of the adrenals and of the spleen, in the first case employing a strong and in the second a weak extract, very nearly the same results were obtained, showing that such a method may have considerable value from a quantitative standpoint. An investigation of the figures shows that nervous tissue obtained from

Table XIX.*

ORGAN EXTRACTED.	Cold solution.	Acid solution.	Boiled solution.
Nervous tissue	10.0	3.0 to 4.0 (blue)	
Liver	8.8 to 9.0	4.1	7.3 to 7.7
Pancreas	8.0	6.0	
First adrenal	8.0		
Second adrenal	7.8	6.8	
First tumor gland	7.6	7.6	7.6 to 8.0
Second tumor gland	7.5	7.5	
Spleen a	7.5	5.0	4.2
Spleen b	7.1 to 7.5		
Lung	5.5 to 6.4		
Kidney	5.0		
Omentum	3.0	2.3	
Fat from omentum (alone)	3.0 to 3.5		
Blood serum from heart	1.5		
Pleural fluid	1.1		
Peritoneal fluid	1.05		

the spinal column has the greatest reducing power in the cold, being slightly higher than that of the tumor, adrenal, spleen, liver and pancreas, all of which are comparatively speaking close to one another. The lungs and kidneys come next, followed by the omentum and other fatty materials which show the smallest reduction of all, indicating clearly that the action in question cannot be attributed to any great extent to the interference of fatty acids. An examination of the figures in the second column shows that after treating with acid, whereby all effects attributable to alkaline solutions and to bodies precipitated by dilute acids are eliminated, the tumor material still produces about the same effect, slightly greater than that of the adrenals, whilst all other organs undergo considerable diminution in reducing power, more especially the extract of nervous tissue, which has fallen to less than one-half of its original strength. The effect of boiling is to produce marked diminution in those cases in which a considerable precipitate is produced.

It must be remembered that the material employed in this series of experiments was obtained at an autopsy, consequently, although not more than a few hours elapsed from the death of the patient to the completion of the experiments, certain post-mortem chemical changes might have intervened sufficient to produce a considerable variation in the reducing power of the individual tissues.

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* These quantities represent the volume in cubic centimeters of a n/500 iodine solution capable of being reduced by the extract of one gram of each of the tissues or sera enumerated in the first column.

The next series recorded in Table XX refers to results obtained on treating the extracts of the organs of normal animals with dilute iodine in the presence of starch.

Table XX.

ORGAN EXTRACTED.	Cold solution.	Acid solution.	Boiled solution.
Normal guinea-pig:			
Brains	5.8	5.3	5.7
Liver	13.0	12.0	12.0
Adrenals	15.0	14.5	14.5
Kidneys	14.6	14.2	14.3
Blood	1.5	1.5	....
Normal rabbit:			
Kidneys	10.3	10.0	*8.0
Adrenals	9.5	9.0	9.4
Liver	8.7	8.0	*5.1
Lung	5.0	4.7	5.5
Fat	1.0	1.0	1.0
Blood	1.4	1.4	....

* Heavy precipitate on boiling.

It may be observed that the results in this series do not differ very materially from those obtained in the human autopsy case referred to above. The point most worthy of notice is the reducing power of the adrenal extracts which in both cases lies very close to that of the kidneys and liver; in the case of the guinea-pig slightly higher, in that of the rabbit slightly lower than that of the kidneys. In both cases the kidneys, adrenals and liver head the list with a comparatively high reducing power, whilst the blood serum and fatty tissue both show an extremely low reduction, from one-seventh to one-tenth of that shown by the kidneys. Such results afford further proof that any differentiation of adrenal tissue or tissue supposedly derived from the adrenals by means of the starch iodine reaction is, from a quantitative standpoint, entirely out of the question.

We further made extracts of a tumor of the stomach and of the neighboring pepsin secreting cells in order to observe whether there was any material difference in reducing power, but none was observed, as will be seen from the figures 2.2 and 2.4 given by different portions of the tumor and 2.2 and 2.3 by unaffected portions of the stomach wall. The table referring to this case and subsequent cases is omitted owing to lack of space, but it may be briefly stated that provided exactly identical conditions are maintained through any series of experiments, no differentiation by this means is, so far as we have at present been able to observe, capable of practical application. The reduction of iodine by means of solutions which have been treated previously with an excess of weak acid is slightly less than that of the original solutions, whilst energetic boiling resulting in a precipitation of proteids frequently results in a very material reduction of the reducing power. Fatty materials cause very small reduction. This is also the case with blood serum. Only in two cases was a result as high as 2.5 to 3 c.c. n/500 iodine solution by means of 1 c.c. of serum recorded; in one case the blood of a normal individual having an exceptionally high specific gravity and nitrogen content and in the second that of a case of cancer of the breast with metastases. The usual result was in the neighborhood of 1.4 to 1.6.

Blood corpuscles and leucocytes in the form of a clot produced a reduction ranging from 2 c.c. to 3 c.c. and it is proposed when time permits to make a series of determinations on the blood as a whole, of a series of cases, especially those in which a good iodinophilia is obtained with leucocytes. In all these cases treatment with acid invariably reduced the results, but never to an extent of more than fifteen or twenty per cent.

Examination of urine. The figures obtained in this series of experiments vary widely, from two to fourteen c.c. of iodine being required per c.c. of urine, but it is to be observed that perfectly constant results were obtained from the same specimen of urine, whilst that of the same individual might vary considerably from day to day. The highest results recorded were obtained in a case of advanced diabetes from urine containing six per cent of sugar. On the other hand, a urine obtained from a second diabetic case in which the sugar content was also above five per cent, showed results very little in excess of the normal. In a series of cases in which an impaired metabolism might have been expected to result in the secretion of an excess of bodies capable of producing this reduction of iodine in urine, this test was carried out, but it is not possible to distinguish such cases from the normal, and it will be necessary to carry out a good deal more work in this direction before recording any noteworthy results. The effect of adding an excess of acid to urines previously slightly alkaline was not found to be as great as might have been anticipated.

We also tried the effect of adding an excess of iodine solution to any given material the reducing power of which was to be determined, allowing the mixture to stand for a couple of minutes, titrating back the excess iodine with a sodium-thio-sulphate solution of equal strength and adding starch just before the end point was reached. Such a method was also capable of giving duplicate results on the same material provided sufficient care was exercised, but considerable variations between the results obtained by this method and those obtained by the direct method were frequently observed.

Examination of tumors of the kidney. A couple of cases of hypernephrom which had been preserved in formalin were submitted to examination following the directions given by Croftan and at the same time a sarcoma similarly treated and kidney tissue were submitted to an identical treatment. In no case was a reduction obtained equal to that of normal tissue, and the fact must not be overlooked that formalin itself if not removed is capable of exerting a reducing action upon iodine starch solution.

Summary. 1. It is possible, employing a n/500 iodine solution and a freshly prepared starch solution, to titrate the extracts of a large ariety of normal tissues provided the greatest precautions are main- ained regarding the uniformity of working conditions. The time actor and the stage at which the end point is recognized are most mportant and should be determined independently by each indi- idual employing the method, such a method having merely a com- arative but no absolute value.

2. The reducing power of an aqueous extract obtained from the drenals is possessed of a strength averaging about the same as that

obtained from the kidneys and liver, the proportions being such that it appears impossible to differentiate between the adrenals and tumors derived from the adrenals and the other organs of the body by Croftan's method.

3. Blood serum, urine, etc., exert a reducing effect upon the solution in question, and it is proposed to accumulate data bearing upon this question with a view to observing whether such a reaction has any value as a means of diagnosing certain pathological conditions.

September, 1903.

Croftan. Virchow's Archiv. Vol. 169.

A NOTE ON THE INFLUENCE EXERTED BY X-RAYS ON THE METABOLISM OF NORMAL ANIMALS.

BY G. H. A. CLOWES, Ph. D., AND W. W. PLUMMER, M. D.

As a preliminary step to the investigation of the influence exerted by X-rays on the metabolism of cancer animals, a series of experiments was carried out on normal animals as follows: Eight guinea-pigs of the same origin and weighing 350 grams apiece were isolated in large funnels provided with a wire gauze base so as to facilitate the collection of urine and fæces. Four of them were exposed to the action of the X-rays, two for forty minutes and the remaining two for twenty minutes every second day for a period of thirty days, the four remaining animals being kept as a check on the experimental animals. The pigs were stretched on their backs on a board in such a manner that the abdomen was exposed to the direct force of the rays at a distance of eight inches from the poles, a current of 4 amperes and 110 volts being employed. For the first eight exposures merely a small portion of the hair of the abdomen was removed, but for the last five experiments the skin was shaved clean. All eight pigs received the same uniform diet of carrots, with occasional addition of greens. They were weighed at the same time daily and determinations made of the nitrogen of the urine and fæces and of the phosphoric acid, chlorides, indican, etc., of the urine. Blood counts were made from time to time, both before and after the exposures. At the end of the month the pigs were killed and their blood subjected to chemical analysis and tissues to pathological examination. The curves representing the increase in body weight show practically no deviations under the influence of the periods of X-ray treatment. At the end of the thirty days the four normal pigs showed an average increase of 40 per cent, the X-ray pigs an increase of 39 per cent of their original body weight. A scarcely greater difference was to be observed in the nitrogen loss in the urine and fæces, the normal pigs showing an average daily loss per kilo of body weight, amounting to 411 millegrams, whilst that of the X-ray pigs amounted to 394 millegrams. It is interesting to note that this figure is practically double that of the normal human being. The excretion of phosphates and indican followed the same lines as that of nitrogen, being slightly lower in the X-ray pigs than in the normal, which would scarcely be the case had the action of the X-rays on these animals been associated with a destruction of tissue. Blood counts showed fairly uniformly from four to five million reds, but considerable variations in leucocytes before and after exposure to the rays. The following table, based on the average of several determinations, shows in five cases out of six a considerable diminution in the number of leucocytes shortly after the completion of an experiment. The precaution was taken of regulating the diet of the animals in such a manner that the variations can not be attributed to that source. The chemical analyses of the blood gave practically identical results on the normal pigs and the X-ray pigs, the chlorine content averaging .3 to .35 per cent, the nitrogen 2.5 to 2.8 per cent, the only recognizable difference being a slightly greater degree of alkalinity of the serum of the X-ray pigs as compared with that of the normal.

TABLE.

Leucocyte count before and after exposure to X-rays.

Before.	After.
8,800	5,800
5,000	3,700
11,500	6,500
6,500	6,500 (?)
6,100	4,000
7,200	5,600

April, 1903.

A NOTE ON THE QUANTITATIVE ESTIMATION OF PHOSPHATES IN STOMACH CONTENTS.

By G. H. A. Clowes, Ph. D. (Published in the American Journal of Pharmacy, Vol. 75. July, 1903. No. 7.)

Summary.

It has been shown that the usual method of roughly estimating the effect due to acid phosphates in the titration of stomach contents is incorrect; that one of the three acid affinities of phosphoric acid is saturated at the alizarin end point, and a second at the phenolphthalein end point. Consequently less than one-half of the total effect due to phosphates lies between the diazo (so called) and alizarin end points; while at least an equal effect is exerted on the portion usually looked upon as combined HCl.

A comparatively simple method has been suggested whereby a fairly accurate estimate of the phosphates present and the effect which they exert may be directly determined, dependent

(1) Upon the transition from NaH_2PO_4 to Na_2HPO_4 between the alizarin and phenolphthalein end points;

(2) The transition from Na_2HPO_4 to Na_3PO_4 between the phenolphthalein end point alone and in the presence of $BaCl_2$, whereby the alkaline Na_3PO_4 is converted into $Ba_3(PO_4)_2$ so soon as formed and thus removed from the scene of action.

THE RELATIONSHIP BETWEEN THE FREEZING-POINT DEPRESSION AND SPECIFIC GRAVITY OF URINE, UNDER VARYING CONDITIONS OF METABOLISM, AND ITS CLINICAL VALUE IN THE ESTIMATION OF SUGAR AND ALBUMIN.

By G. H. A. Clowes, Ph. D. (Published in the American Journal of Physiology, Vol. 9. July 1, 1903. No. 5.)

Summary.

1. In normal urine the depression of the freezing point is directly proportional to the specific gravity, and may be determined from the latter by multiplying the figures after the decimal point by 75. (As an example, if the specific gravity is 1.022, $0.022 \times 75 = 1.65$, which is found by experiment to be the depression of the freezing point of such a solution in degrees C.) (See original article, Tables I and II.) This portion of the work is in agreement with the findings of Fuchs.

2. In pathological urines other than those containing sugar and albumin, the same tendency to maintain a constant proportion between the freezing point and specific gravity may be observed, although within rather wider limits than in the case of normal urines. (See original article, Tables III and IV.)

3. The average weight of the molecule or ion in urine may be determined from these figures as approximately 56 to 60, making use of Beckmann's formula,

$$M = E \frac{m}{\Delta},$$

where M = molecular weight of substance dissolved; E = a constant having the value of 18.5 when water is employed as the solvent;

m = the quantity of substance dissolved, assumed in this case to be 2.33 grams of substance for each 0.01 of specific gravity; and Δ = the observed depression of the freezing point (0.75° for each 0.01 of specific gravity).

4. In cancer, typhoid fever, and other diseases in which the excretion of chlorides is extremely small, the effect exerted upon the freezing point depression is not so great as might be expected from the removal of such a large proportion of the smaller ions. This tendency to maintain a constant state of equilibrium in the average size of the ions and molecules present in the urine may account for the high retention of the products of sub-oxidation and abnormal metabolism, known to exist in these cases. This view of the case may be advanced as a further argument in favor of the therapeutic administration of chlorides to patients on a milk diet, in order that molecular equilibrium of the urine may be maintained, and a more complete elimination of the products of metabolism effected.

5. In diabetic urines, the quantity of sugar may be very readily estimated by determining the lowering of the freezing point of the urine in question, and calculating the theoretical lowering of the freezing point from the specific gravity. The difference between these two quantities in degrees C., when multiplied by 6, gives a very close approximation of the actual percentage of sugar present in the solution. This factor, 6, calculated on a theoretical basis and subsequently confirmed in practice, is dependent on the fact that the molecular weight of sugar is 180, whilst the average of urine solids is below 60. (See original article, Tables VI, VII, VIII, IX, X, and XI.) These results are not all in accord with the observations made by Fuchs, who arrived at his factor, 10, by purely empirical means.

6. The quantity of albumin may be estimated by determining the specific gravity and freezing point in a urine to which a couple of drops of dilute acetic acid has been added, then boiling in order to precipitate the albumin, filtering and once more determining the specific gravity and freezing point. The second specific gravity is reduced to the same ionic concentration as the first, by multiplying its decimal portion by the freezing point before boiling, and dividing by the freezing point obtained after boiling. This calculated spe-

cific gravity is then subtracted from the original specific gravity determined before boiling, and the difference multiplied by the factor 400, which gives the percentage of albumin. (See original article, Table XII.)

7. In a urine containing both albumin and sugar, the former is first estimated by addition of acetic acid, as above, and the freezing point and specific gravity obtained after boiling are employed in the estimation of sugar.

THE METACHROMATISM OF MAST CELL GRANULES AND MUCIN.

By G. H. A. Clowes, Ph. D., and Alice G. Owen. (Published in the Journal of Medical Research. 1904.)

Summary.

The metachromatism exhibited by the granules of mast cells on staining with 10 per cent polychrome methylene blue may be considered as a simple acid-alkali reaction, the compound formed between the methylenazur of the stain and the granules being sufficiently sensitive to acids and alkalis to function as an indicator in the strict chemical sense of the word.

The granules exhibit a *blue coloration with acids* and a *red with alkalies.*

The tendency to change color on passing from acid to alkaline solutions is exhibited by other constituents in the tissue but to a considerably less marked degree.

Whilst the transition from blue to red with alkalies and from red to blue with acids may be observed whatever the nature of the hardening agent previously employed, it should be noted that formalin hardened material shows a far greater tendency to blue and alcohol and sublimate-hardened material to red, even though the original hardening solutions were perfectly neutral. The ability of formalin to combine with ammonia and amido derivatives may well account for this peculiarity.

Oxidizing and reducing agents produce no marked color changes from red to blue or vice versa when stained mast cells are submitted to their action. Reducing agents tend to decolorize the sections as might be anticipated.

The granules are insoluble in weak acids but readily soluble in weak alkalies. Their tendency to dissolve may, however, be ma-

terially affected by their preliminary treatment with various hardening agents and mineral salts.

The globules of mucin present in goblet cells of the intestina mucosa stain faintly blue with dilute polychrome methylene blu and show a tendency to red on treatment with acids. On heavil overstaining with concentrated polychrome methylene blue and decolorizing with acetic acid the globules are stained red and on subsequent treatment with dilute alkalies are decolorized or rendere slightly blue. The red color may once more be restored by subsequent treatment with dilute acid, but is not so intense as in th original section. The globules in question are also considerabl more soluble in alkalies than are the granules of mast cells.

The leucocytes on staining with polychrome methylene blue sho much less polychromatic tendency than the mast cells, retaining bot in acid and alkaline solutions a violet to blue color up to the point o decolorization.

Since mast cells take polychrome methylene blue much mor readily than does mucin and exhibit a blue color with acids and red with alkalies, whilst mucin is red with acids and decolorize with alkalies; also since the globules of mucin are far more solubl in alkaline solutions than are the mast-cell granules, it may be concluded that they differ very materially in chemical constitution.

Much is already known regarding the effect exerted on the staining properties of bodies of the type of thionin, methylene blue, etc. on introducing acids or basic groups at various points in the molecule; also regarding the effect of acids, alkalies and oxidizing an reducing agents on the compounds thus formed.

A thorough investigation of the nature of polychrome methylen blue from a purely chemical standpoint, following the lines adopte by Michaelis, would be extremely valuable. It should then be possible, making use of the various constituents of this stain unde varying conditions of acidity and alkalinity, to obtain considerabl information regarding the chemical nature of the granules and othe secretory products present in mast cells, leucocytes, etc., and to compare them with bodies of known chemical composition.

This line of research is one which we propose to take up at th first available opportunity and as soon as the stains in question ar procurable.

THE THEORY OF INDICATORS AND ITS BEARING ON THE ANALYSIS OF PHYSIOLOGICAL SOLUTIONS BY MEANS OF VOLUMETRIC METHODS.

By G. H. A. Clowes, Ph. D. (Published in the American Journal of Pharmacy. 1904.)

In this paper the physico-chemical theory of indicators was discussed in so far as its principles were found to be applicable to the solution of problems involved in the volumetric analysis of physiological solutions.

Practically all indicators are sensitive to strong acids and strong bases. They may, however, be divided into three distinct classes when tested with a series of weak acids and weak bases of the type present in physiological solutions. At one end of the scale are those which in view of an extremely weak acid affinity are particularly affected by weak acids and are, comparatively speaking, indifferent to weak bases, whilst at the other end of the scale are to be found those which are possessed of strong acid or weak basic combining groups which render them very susceptible to the influence of weak bases but more or less indifferent to that of weak acids.

It is further possible to differentiate between a series of two or more acids or bases present in the same solution, provided one can find indicators, the combining affinity of which to form neutral salts, lies intermediate between those of the bodies to be titrated. A good illustration of this mode of differentiation is the recognition of the three acid groups of phosphoric acid, the first one of which coincides with the first alizarin end point, the second with the second alizarin end point, or with the phenolphthalein end point, and the third with the phenolphthalein end point in the presence of barium chloride;

or aspartic acid, for example, the first acid affinity of which is neutralized at the alizarin or phenolphthalein end point, the second only at the end point of Porrier's blue. Carbonic acid affords an interesting contrast to aspartic acid, neither of its acid groups being strong enough to affect alizarin, one only giving an indication with phenolphthalein and both with Porrier's blue. It will thus be seen that in their behavior towards phenolphthalein and alizarin we have a sharp differentiation between the strongest acid affinities of aspartic and carbonic acid respectively.

At the other end of the scale phloroglucinvanillin is sensitive to the amido group present in aspartic acid and entirely indifferent to the acid groups referred to above. Whilst Porrier's blue is absolutely indifferent to ammonia and all weak basic groups, alizarin is sensitive to ammonia but not to amido groups of the type present in glycocol, leucin and tyrosin. Phloroglucinvanillin as an indicator gives a sharp titration of NH_2 groups of this class and one of the NH_2 groups in asparagin. It is unaffected by the second or amido group of asparagin and those of the same type present in acetamid, formamid, urea, etc.

In discussing the effect of albumoses, peptones, etc., on indicators, it was shown that perfectly pure preparations of these substances show a wide range of variation in titration end points, attributable presumably to the influence of weak basic groups present in the molecule; further, that in advancing hydrolysis the range from the lowest to the highest end points was increased, and that this effect cannot be attributable to the influence of phosphates, organic acids and chlorides to the same extent as has been generally supposed.

The comparison of volumetric with gravimetric analyses of normal and pathological stomach contents showed that provided a plain Ewald, or by preference a purely proteid test meal were employed, and the materials examined in the space of three hours, with very few exceptions the phenolphthalein end point gives a fairly accurate estimate of the total available hydrochloric acid present in the stomach, either in a free state or combined more or less feebly with proteids. The influence exerted by phosphates is usually small and may be allowed for by making a separate estimation of the quantity present according to a method suggested by the writer in a previous paper. The end point of phloroglucinvanillin drops used on a warm plate gives a fairly accurate estimate of the actual free hydrochloric acid. The range of titration from the phloroglucinvanillin end point to that of phenolphthalein, after allowing for the influence of phosphates and traces of organic acid may be said to be a function of the basic affinities of proteids and their decomposition products present in the solution. The total range and the end points of intermediate indicators are dependent upon the total quantity of such bodies present in the solution and the extent of their hydrolysis or decomposition, and provided comparable conditions of experiment are employed this range may be said to be fairly proportionate to the peptic activity of the mixture.

Under pathological conditions where hydrochloric acid is absent r diminished in quantity, and peptic digestion fails to take its nornal course, especially where organic acids have been produced under he influence of bacteria, the above rules are no longer applicable. The total range no longer bears the slightest relationship to the titraion results; chlorides determined gravimetrically are found to be ar below the phenolphthalein end point; the nitrogen present in o-called phosphotungstic filtrate rises above the normal, which is rom 20 to 25 per cent of the total nitrogen in a well-digested mixure. All these factors point to the abnormal breaking down of proeids beyond the point normally reached in peptic digestion in the tomach, presumably under the influence of bacterial enzymes funcioning in a neutral solution with the liberation of additional weak asic and acid groups, as a result of the abnormal hydrolysis.

In tryptic digestion due allowance must be made for the influence f a known quantity of sodium carbonate which may be estimated ndependently. Both the acid groups of this body are capable of xerting an influence upon Porrier's blue, whilst only one affects henolphthalein and other indicators such as alizarin or phlorolucinvanillin are entirely unaffected. After allowing for the influnce due to carbonic acid it is found that the range of titration ncreases with increasing digestion, being at the early stages more r less proportionate to the amount of proteid brought into solution as estimated by the total nitrogen, but subsequently increasing in undue proportion to the total nitrogen as digestion proceeds, with the formation of simpler nitrogenous products.

The bearing which work of this nature may have on the solution of certain problems connected with the constitution of proteids and their decomposition products was also discussed. Titrations of normal blood serum showing a wide variation with different indicators which could not possibly be attributed to the inorganic constituents of the serum were tabulated and the necessity of an agreement upon certain specific indicator end points for serum titration emphasized.

The titration results on urine were shown to be fairly dependent on the amount of phosphates present, the small proportion of amido bases and weak acids present exerting a relatively small effect in normal cases. A normal urine should be acid to phenolphthalein and alkaline to alizarin, the end point lying somewhere between the neutralization points of these indicators. In other words, whilst the first acid group of the phosphates should be neutralized, the third acid group should remain free, varying proportions of the second group being neutralized or not according to the state of equilibrium of the system. Naturally in the presence of any considerable quantity of ammonia which in contradistinction to urea exerts a powerful effect upon alizarin and phenolphthalein, the urine would be less acid, or even alkaline to phenolphthalein and more strongly alkaline to alizarin than is normally the case.